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Determinants of Resting Metabolic Rate and Factors Contributing to Erroneous Measurements in the Female Athlete

Submitted by

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BSc, MSc, RD

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Declaration of Authorship & Sources

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgement in the main text of the thesis. All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees.

Megan A. Kuikman Date: December 24, 2024

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List of Publications Related to Thesis

1. **Kuikman, M.A.**, McKay, A.K.A., Minahan, C., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Smith, E.S., McCormick, R., Tee, N., Skinner, J., Ackerman, K.E., and Burke, L.M. (2024). Effect of menstrual cycle phase and hormonal contraceptives on resting metabolic rate and body composition. *International Journal of Sport Nutrition and Exercise Metabolism, 34*(4), 207-217. doi: 10.1123/ijsnem.2023-0193

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This manuscript is currently in review in the Journal of Sports Science with the included manuscript in chapter 8 having undergone revisions.

List of Conference Presentations

2024 **Kuikman, M.A.,** McKay, A.K.A., McCormick, R., Tee, N., Vallance, B., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. The temporal effects of altitude and low energy availability manipulation on resting metabolic rate in female athletes. *Abstract for oral presentation at the 2024 Women in Sport Congress, Sydney, Australia, March 2024.*

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Additional Publications during the Candidature

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2024 Smith, E.S., Weakley, J., McKay, A.K.A., McCormick, R., Tee, N., **Kuikman, M.A.,** Harris, R., Minahan, C., Buxton, S., Skinner, J., Ackerman, K.E., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. (2024). Minimal influence of menstrual cycle or hormonal contraceptives on performance in female rugby league athletes. *European Journal of Sports Science, 24*(8):1067-1078.

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Abstract

Resting metabolic rate (RMR) represents the energy required to maintain the systems of the body and to regulate body temperature at rest. RMR is measured using indirect calorimetry with measurements occurring in an overnight fasted and rested state. Traditionally, an athlete's RMR has been measured as part of the estimate of total daily energy requirements. A more novel potential use of RMR measurements is as an indicator of low energy availability (LEA) with energy availability representing the energy left over for the body after accounting for the energy expended through exercise. A decreased RMR is a proposed potential indicator of LEA and signals that insufficient energy is being partitioned to one or more systems underpinning body function. However, RMR measurements are not currently supported for use as an indicator of LEA due in part to high measurement variability. There are numerous biological, technical and environmental factors that contribute to variability in RMR measurements as well as factors that may lead to erroneous RMR measurements. This thesis will investigate factors contributing to variability in RMR measurements as well as artifacts that may interfere with a valid and clinically useful interpretation of the data.

Study 1: Effect of menstrual cycle phase and hormonal contraceptives on resting metabolic rate and body composition

The purpose of this study was to determine if RMR changes across the menstrual cycle (MC) and differs compared to hormonal contraceptive (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. There was no effect of MC phase on

absolute RMR (p=0.875) or relative RMR (p=0.958) nor was there an effect of HC use on absolute RMR (p=0.068) or relative RMR (p=0.309).

Study 2: The temporal effects of altitude and low energy availability manipulation on resting metabolic rate in female race walkers

This study investigated the temporal effects of ~1800 m altitude exposure and energy availability (EA) manipulation on RMR. Twenty elite female race walkers underwent a 3-week training camp at an altitude of ~1800 m. During the first two weeks, athletes consumed a high EA (HEA) diet of 45 kcal·kg fat free mass $(FFM)^{-1}\cdot day^{-1}$. During the final week, half the athletes consumed a low EA (LEA) diet of 15 kcal·kg $FFM^{-1}\cdot day^{-1}$ while the others continued on a HEA diet. Athletes in the HEA group had RMR measured at baseline (~580 m) prior to altitude exposure (Pre-alt), at 36 hours (36h-alt), 2 weeks (Wk2-alt) and 3 weeks into altitude exposure (Wk3-alt), and at 36 hours post-altitude exposure at ~580 m (36h-post). Athletes in the LEA group underwent RMR measurements at Pre-alt and before (Wk2-alt) and after the 7 days of LEA (Wk3-alt). Compared to Pre-alt, the RMR of HEA athletes was increased at 36h-alt (+5.3±3.1%; p=0.026) and Wk2-alt (+4.9±4.9%; p=0.049) but was no longer elevated at Wk3-alt (+1.7±4.2%; p=0.850). The RMR of HEA athletes at 36h-post was lower than all timepoints at altitude ($p<0.05$), but was not different from Pre-alt (-3.9±7.2%; p=0.124). The 7-day period of LEA exposure at altitude did not affect RMR (p=0.347).

Study 3: Impact of acute dietary and exercise manipulation on next day RMR measurements and DXA body composition estimates

This study investigated the effects of acute diet and exercise manipulation on RMR measurement variability. 10 male and 10 female endurance athletes (12 cyclists, 5 triathletes, 4 runners) of Tier

2 (n=18) to Tier 3 (n=2) calibre underwent five conditions using a Latin square counterbalance design. For 24 hours, athletes consumed a diet providing excessive energy availability (75 kcal·kg FFM^{-1}) without exercise, high energy availability (45 kcal·kg FFM^{-1}) without or with exercise, or low-energy availability (15 kcal·kg FFM^{-1}) without or with exercise. There were no sex differences in relative RMR (p=0.158) nor effects of any of the five conditions on RMR (p=0.358).

Study 4: Barriers and enablers to measuring resting metabolic rate in the high-performance sporting system: A qualitative exploratory study

This study investigated barriers and enablers to measuring RMR in real-world, high-performance sport environments. Twelve semi-structured interviews were conducted with RMR technicians (n=6 dietitians, n=6 physiologists) employed across six National Institute Networks, two National Sporting Organisations and one professional sporting code. Barriers included lack of confidence in measuring RMR, burden of measurement on athlete and technician, confusion over measurement responsibility, and scepticism in RMR measurements as an indicator of LEA. Subthemes that contributed to scepticism included: the RMR thresholds used to indicate LEA, unanswered research questions, and measurement errors introduced by athlete presentation, testing equipment and/or environment. Enablers to use of RMR measurements included perceived value of RMR measurements as a "piece of the puzzle" when assessing for LEA and its use as a longitudinal measure.

Summary and future direction:

This series of research studies investigated factors contributing to variability and error in RMR measurements. As a result, several gaps in the literature have been addressed and results provide

novel insight, including: 1) MC phase does not contribute to variability in RMR measurements in female athletes 2) HC usage does not contribute to variability in RMR measurements in female athletes 3) RMR is acutely increased with low altitude exposure but returns to baseline values with more prolonged exposure in female athletes 4) 7 days of LEA while at altitude does not impact RMR in female athletes 5) Large variations in EI does not introduce error into next day RMR measurements 6) Prolonged continuous cycling at moderate intensity does not introduce error into next day RMR measurements 7) While RMR measurements are seen by practitioners working with the high-performance athlete as a piece of the puzzle when assessing for LEA and valuable when used longitudinally, there are multiple barriers that need to be addressed when measuring RMR in the high-performance sport environment. A uniform approach is needed when measuring RMR both within the high-performance sport environment and research so that measurement variability is minimised. While this thesis addressed some notable gaps in the literature, further research is needed This includes assessing the impact of training status independent of LEA on RMR, and if resistance exercise and high intensity exercise needs to be restricted the day prior to a RMR measurement. A normative database of RMR in athletic cohorts is also needed that considers characteristics that impact the RMR to FFM relationship to better understand what a normal RMR is, and what threshold may indicate LEA. This could also provide insight into novel ways of assessing an athlete's RMR, such as indexing RMR to regional estimates of FFM.

List of Abbreviations and Nomenclature

ABM	Adjusted body mass
AIS	Australian Institute of Sport
BM	Body mass
BMI	Body mass index
BMR	Basal metabolic rate
CAT2	Clinical assessment tool version 2
CHO	Carbohydrate
COC	Combined-monophasic version of the oral contraceptive pill
COM-B	Capability, Opportunity, Motivation- Behaviour
CO_2	Carbon dioxide
CV	Coefficient of variation
DMPA	Depot-medroxyprogesterone acetate
DXA	Dual energy x-ray absorptiometry
EA	Energy availability
EEE	Exercise energy expenditure
EI	Energy intake
EPOC	Excess post-exercise oxygen consumption
FARC	Female athlete research camp
FM	Fat mass
FFM	Fat free mass
GXT	Graded exercise test

HB	Harrie-Benedict
HC	Hormonal contraceptive
HEA	High energy availability
HIIT	Hight intensity interval training
IGF-1	Insulin-like growth factor 1
IOC	International Olympic Committee
LBM	Lean body mass
LDL	Low density lipoprotein
LEA	Low energy availability
LEAF-Q	Low Energy Availability in Females Questionnaire
LH	Luteinising hormone
LSC	Least significant change
MC	Menstrual cycle
MET	Metabolic equivalent
NC	Naturally cycling
NIN	National institute network
NSO	National sporting organisation
OCP	Oral contraceptive pill
O_2	Oxygen
PPO	Peak power output
REDs	Relative energy deficiency in sport
RMR	Resting metabolic rate
SMR	Sleeping metabolic rate

TC	Total cholesterol
TDF	Theoretical Domains Framework
TEF	Thermic effect of food
T3	Triiodothyronine
uN2	Urinary nitrogen
USG	Urine specific gravity
VO_2 max	Maximal aerobic capacity
VO_2	Oxygen consumption
VCO2	Carbon dioxide production

Glossary of Terminology

Adaptable low energy availability	Reduction in energy availability associated with benign effects and minimal or no impact on long-term health, well-being or performance
Basal metabolic rate	Energy required to maintain the systems of the body and to regulate body temperature at rest measured under inpatient conditions
Energy availability	Dietary energy left over and available for optimum function of body systems after accounting for energy expended from exercise
Excess post-exercise oxygen consumption	Prolonged increase in oxygen uptake in the recovery period after exercise
Exercise energy expenditure	Additional energy expended above that of daily living during exercise
Low altitude	1000-2000 m elevation
Menstrual irregularities	Perturbations of the eumenorrheic menstrual cycle, such as amenorrhea, anovulation, or oligomenorrhea
Moderate altitude	2000-3000 m elevation
Moderating factors	Characteristics of individual athletes, their environment, or behaviour/activities that may amplify or attenuate the effect of low energy availability on body systems

Naturally cycling	Women who experience menstruation with a menstrual cycle length of 21-35 days, but ovulation has not been confirmed
Phase 1 of menstrual cycle	Onset of bleeding until day 5 when estrogen and progesterone levels are low
Phase 2 of menstrual cycle	14-26 hrs prior to ovulation and the LH surge with estrogen higher than phase 1, 3, and 4 and progesterone higher than phase 1, but <6.36 $nmol \cdot L^{-1}$
Phase 4 of menstrual cycle	Seven days after ovulation has been confirmed with estrogen levels that are higher than phase 1 and 3, but lower than phase 2, and progesterone >16 $nmol \cdot L^{-1}$
Problematic low energy availability	Reduction in energy availability associated with greater disruption of various body systems and representing a maladaptive response
Relative energy deficiency in sport (REDs)	Syndrome of impaired physiological and/or psychological functioning experienced by male and female athletes caused by problematic low energy availability exposure
REDs Clinical Assessment Tool- V.2	Clinical tool to identify current severity and/or future risk of REDs using accumulation of primary and secondary indicators of REDs

REDs CAT primary indicators	Outcome parameters consistently resulting from problematic low energy availability with the greatest measurement validity and scientific evidence
REDs CAT secondary indicators	Outcome parameters with some scientific evidence resulting from problematic low energy availability with lower measurement validity
REDs CAT potential indicators	Outcome parameters lacking robust scientific evidence but may possibly be linked to problematic low energy availability
Relative RMR	RMR measured by indirect calorimetry divided by an athlete's fat free mass
Resting metabolic rate	Energy required to maintain the systems of the body and to regulate body temperature at rest measured under outpatient conditions
RMR ratio	RMR measured by indirect calorimetry divided by the RMR predicted from an equation
Thermic effect of food	Increased metabolism after a meal due to the energy expenditure of processing and storing food

1 Introduction

Basal metabolic rate (BMR), also known as basal energy expenditure, represents the minimal energy cost of living (Hulbert & Else, 2004). BMR makes up one of the components of total daily energy requirements alongside the thermic effect of food (TEF), exercise activity thermogenesis, and non-exercise activity thermogenesis (Trexler et al., 2014). In the general population, BMR makes up the largest component of total energy expenditure (Trexler et al., 2014). However, compared to the average adult, an athlete's BMR will make up a smaller contribution of total energy expenditure due to increased exercise activity thermogenesis (Manore, 2021). BMR is measured in the morning with the individual staying overnight in the laboratory (Manore, 2021). The measurement of metabolic rate in an outpatient setting, with the individual sleeping outside of the laboratory and commuting to the laboratory for testing, is known as resting metabolic rate (RMR) (Manore, 2021). Measurements of RMR are typically higher than BMR, but similar results are seen when there is an adequate period of rest prior to the RMR measurement (Bone & Burke, 2018; Turley et al., 1993). Because it is more common, particularly within athlete settings, to measure metabolic rate in outpatient conditions, the term RMR will be used throughout the remainder of this review unless discussing a study that has specifically measured BMR.

Although RMR can be estimated using a number of predictive equations, most of the available equations have excluded athletes in their analysis or only included a small number of athletes (Schofield et al., 2019). Investigations of the application of these predictive equations to athletic populations have found that they tend to underestimate the RMR of both male (Carlsohn et al., 2011; Jagim et al., 2018; Thompson & Manore, 1996) and female athletes (Jagim et al., 2018; Thompson & Manore, 1996), potentially due to differences in body composition compared to the

general population and within different athletic populations (i.e. throwers vs. long-distance runners). In support of this, equations that provide the most accurate results in both male (Ten Haaf & Weijs, 2014; Thompson & Manore, 1996) and female athletes (Jagim et al., 2018; Ten Haaf & Weijs, 2014; Thompson & Manore, 1996), require knowledge of an athlete's lean body mass (LBM) or fat free mass (FFM) (Cunningham, 1980, 1991). Given the inaccuracies of predictive equations in athletic populations, when an accurate RMR value is required, laboratory testing is recommended. The gold standard for measuring RMR is direct calorimetry, which measures total heat loss from the body by placing an individual in a thermically isolated chamber from which heat dissipation can be measured (Ferrannini, 1988). However, access to the facilities needed for these assessment methods are incredibly rare, and instead indirect calorimetry is more commonly used (Kenny et al., 2017). Indirect calorimetry assesses the amount of heat generated indirectly according to substrate use and by-product production, by measuring the amount of oxygen consumed (VO_2) and carbon dioxide (VCO_2) produced through respiratory gases (Haugen et al., 2007). By measuring respiratory gases, total energy expenditure can then be calculated using the modified Weir equation (Weir, 1949):

$$\text{Daily energy expenditure} = ([3.94 \times VO_2] + [VCO_2 \times 1.11] + [uN2 \times 2.17]) \times 1440$$

As the urinary nitrogen (uN2) component only contributes to a small value to the calculation of energy expenditure, it is typically excluded (Ferrannini, 1988). Methods of indirect calorimetry include the Douglas bag method, a metabolic cart, or whole-body room calorimetry (Levine, 2005).

Traditionally, RMR has been measured to provide information on an athlete's energy requirements, which can be integrated into nutrition advice and diet plans provided to the athlete.

A more novel use of RMR measurements is as a potential screening tool for exposure to low energy availability (LEA). Energy availability (EA) represents the energy left over for the body after accounting for the energy expended through exercise and is operationally defined as followed (Mountjoy et al., 2023):

$$EA = \text{Energy Intake (EI)} - \text{Exercise Energy Expenditure (EEE)} \div \text{FFM}$$

The negative consequences of LEA exposure were first described within the "Female Athlete Triad" model; an interrelated syndrome of disordered eating, absence of menses or irregular menstrual cycles, and poor bone health (Nattiv et al., 1994; Yeager et al., 1993). Later, the more comprehensive terminology of Relative Energy Deficiency in Sport (REDs) was introduced by the International Olympic Committee (IOC) as a syndrome of impaired physiological function caused by exposure to LEA that can occur in both male and female athletes (Mountjoy et al., 2014, 2018, 2023). Within the REDs model, a decreased RMR is a proposed potential indicator of problematic LEA exposure and signals that insufficient energy is being partitioned to one or more systems underpinning body health or function (Mountjoy et al., 2014, 2018, 2023). A suppressed RMR can be assessed by reporting the ratio of an athlete's measured RMR to the RMR predicted (RMR ratio) from one of the widely available equations, with a RMR ratio <0.90 often being used to indicate a problematic suppression associated with LEA (Sterringer & Larson-Meyer, 2022). However, the RMR ratio that best indicates a maladaptive response to LEA exposure may change depending on the RMR predictive equation used (Alcantara et al., 2024; Strock et al., 2020). Alternatively, an athlete's measured RMR relative to FFM (relative RMR) may be used to assess a suppressed RMR, with a relative RMR <30 kcal·kg FFM^{-1}·day^{-1} often being used to indicate LEA (Loucks et al., 2011; Westerterp, 2003).

The 2023 IOC REDs Clinical Assessment Tool-V.2 (CAT2) provides a framework to assess an athlete's risk or severity of REDs by scoring primary and secondary indicators of LEA (Stellingwerff et al., 2023). The measurement of RMR has been listed as a potential indicator within the REDs CAT2 (Stellingwerff et al., 2023) and as such, is not currently involved in the official risk assessment of REDs. This lack of confidence in the diagnostic value of RMR measurements partly reflects the observations of their poor validity and high variability (Stellingwerff et al., 2023). Indeed, there is a 3-5% intra-individual day-to-day variability in RMR with up to 10% variability over weeks to months (Compher et al., 2006). Before RMR measurements can be used as a surrogate marker of LEA, factors contributing to this variability must be assessed and sources of error eliminated. This review will highlight factors that may contribute to variability in RMR measurements as well factors that may lead to erroneous RMR measurements.

2 Literature Review

2.1 Biological sources of variability

Biological sources of variability represent determinants of RMR (see Figure 2.1). This includes factors that contribute to both intra- and inter-individual variability and may influence RMR independent of EA. Each source of variability will be discussed in a sub-section below.

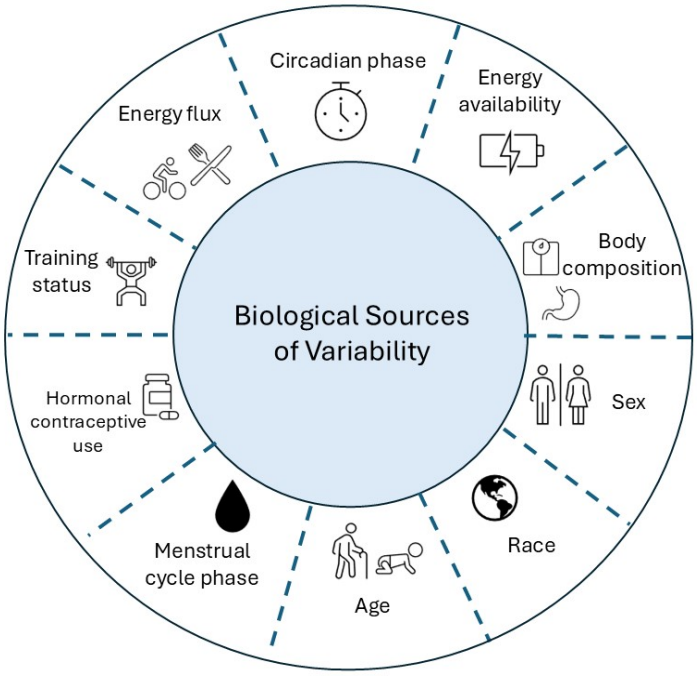

Figure 2.1. Potential biological sources of variability in RMR measurements.

2.1.1 Energy availability

A large increase in EA may induce changes in RMR due to increases in body mass (BM) since BM and its metabolically active components are the major determinants of RMR (Müller & Bosy-Westphal, 2013; Westerterp, 2013). Yet, several studies have shown increases in RMR with exposure to an energy surplus, beyond what can be fully explained by changes in BM (Harris et al., 2006; Kush et al., 1986; Tremblay, Despres, et al., 1992). However, this is not a universal

finding (Dirlewanger et al., 2000; Leibel et al., 1995; Ravussin et al., 1985; Roberts et al., 1990, 1996; Weststrate & Hautvast, 1990) and may relate to differences in the duration and magnitude of hypercaloric intake. Increases in RMR with large increases in EA may further be confounded by residual TEF leading to a falsely elevated RMR (see section 2.3.1). Of more relevance to this review is the impact of LEA on RMR. In non-athletic populations, a reduction in RMR with energy restriction, independent of changes in BM or FFM has been reported and is referred to as adaptive thermogenesis (Doucet et al., 2001; Fothergill et al., 2016; Martin et al., 2022; Müller et al., 2015; Rosenbaum et al., 2008). In athletic populations, the impact of EA on RMR will be reviewed by looking at studies that have measured RMR 1) In athletes with and without indicators of LEA 2) following an intervention that increased EA in athletes with indicators of LEA and 3) following an intervention that implemented a period of LEA.

2.1.1.1 RMR in athletes with and without indicators of LEA

In athletic cohorts, evidence of a suppressed RMR in athletes with indicators of LEA was first demonstrated in female athletes with menstrual dysfunction, with findings that long-distance runners with amenorrhea had a lower relative RMR (25 kcal·kg FFM^{-1}·day^{-1}) compared to eumenorrheic long-distance runners (31 kcal·kg FFM^{-1}·day^{-1}) and sedentary eumenorrheic controls (32 kcal·kg FFM^{-1}·day^{-1}) (Myerson et al., 1991). Since this publication in 1991, 18 other studies have compared the RMR of eumenorrheic athletes to athletes with various forms of menstrual dysfunction (i.e. amenorrhea, oligomenorrhea, anovulatory cycles and luteal phase defects) with 14 of these studies reporting a lower RMR in athletes with menstrual dysfunction (De Souza et al., 2007; Doyle-Lucas et al., 2010; Fahrenholtz et al., 2018; Kaufman et al., 2002; Koehler et al., 2016; Lebenstedt et al., 1999; Melin et al., 2015; Myburgh et al., 1999; Myerson et al., 1991; O'Donnell et al., 2009; Reed et al., 2015; Singhal et al., 2014; Strock et al., 2020; Tornberg et al.,

2017). While this provides strong evidence for a suppressed RMR in female athletes with menstrual dysfunction, it should be noted that many studies implemented poor methodological control when characterising the menstrual status of athletes according to Best Practice Guidelines (Elliott-Sale et al., 2021). As such, it is possible that there was underlying menstrual dysfunction in female athletes classified as eumenorrheic, or that the menstrual dysfunction observed was not due to LEA (i.e. amenorrhea due to polycystic ovarian syndrome). Only four other studies have compared the RMR of athletes with and without other indicators of LEA. One of these studies reported no difference in the RMR of female cyclists at high or low risk of LEA based on responses to the Low Energy Availability in Female Questionnaire (LEAF-Q) (Jurov et al., 2020). The three remaining studies used calculations of EA based on estimations of EI and EEE to indicate LEA. Using this approach, an EA <30 kcal·kg FFM^{-1}·day^{-1} is typically defined as LEA and 30-45 kcal·kg FFM^{-1}·day^{-1} as "suboptimal" EA (Burke et al., 2018). However, these results must be interpreted with caution as there are numerous difficulties associated with the calculation of EA that may result in residual error (Burke et al., 2018). Nonetheless, one study of female athletes found that those with a reduced EA of ~39 kcal·kg FFM^{-1}·day^{-1} or low EA of ~19 kcal·kg FFM^{-1}·day^{-1} had a lower RMR than those with an optimal EA of ~52 kcal·kg FFM^{-1}·day^{-1} (RMR~ 28 vs. 31 kcal·kg FFM^{-1}·day^{-1}) (Melin et al., 2015). A study of female teenage runners (Age: 17 years) found no difference in the RMR of those with an EA above or below 30 kcal·kg FFM^{-1}·day^{-1} as well as no association between EA and RMR, but when reanalysing with a different threshold, those with an EA >45 kcal·kg FFM^{-1}·day^{-1} had a greater RMR than those with EA below this threshold (Kinoshita et al., 2021). Finally, there was no difference in the RMR of male triathletes and cyclists with an EA above or below 30 kcal·kg FFM^{-1}·day^{-1} (Jurov et al., 2021), noting that this is the only available study of male athletes (Jurov et al., 2021). Evidently, while there is evidence to support

a reduced RMR in athletes with menstrual dysfunction, there is little evidence to support a decreased RMR in athletes presenting with other indicators of LEA. Notably, menstrual dysfunction results from prolonged exposure to LEA whereas the other indicators of LEA may reflect acute LEA exposure that is of insufficient duration or magnitude to result in a reduced RMR.

2.1.1.2 RMR in response to increased EA in athletes with indicators of LEA

Of the three available investigations of nutrition interventions to increase EA in athletes with indicators of LEA, two involved case studies (see Table 2.1). Two reports involved an increase in the EA of female athletes with amenorrhea, with a case study describing an increase in RMR relative to LBM and restoration of menses in two athletes (Mallinson et al., 2013). Meanwhile, a more conventional intervention study found no effect on the RMR of athletes despite weight gain and restoration of menses (Guebels et al., 2014). The final study involved a case history of a wheelchair badminton athlete who presented with a suppressed RMR ratio (Flueck & Koehler, 2022). This athlete increased her EI by 200-400 kcal·day^{-1} and completed two additional arm crank exercise sessions per week for a year, with the outcome of a RMR ratio >1.0, despite a 11 kg decrease in fat mass (FM) (Flueck & Koehler, 2022). However, this study must be interpreted with caution as cutoff values to indicate a suppressed RMR were derived from literature on able-bodied athletes (Strock et al., 2020). Further investigation of the effect of increased EI on athletes with REDs is needed, including the time-course of the recovery of a suppressed RMR and its relationship with the recovery of other biological systems impacted by LEA.

Table 2.1. Studies examining the impact of increased EA on RMR in athletes with indicators of LEA.

Reference	Population	RMR protocol	Intervention	Results
Mallinson et al., 2013	n= 2 females with amenorrhea Engaging in >7 hrs/wk of exercise	Metabolic cart (SensorMedics Vmax) with hood Overnight fasted with >24 hrs exercise restriction 30-45 min rest, 30 min measurement with steady state to calculate RMR Body composition via DXA	Increased EI by 20-30% above baseline TEE	Case 1: Baseline RMR ~27 kcal·kg LBM^{-1}·day^{-1}; Month 12 RMR ~33 kcal·kg LBM^{-1}·day^{-1} 4.2 kg increase in BM Mense resumed after 2.5 months Case 2: Baseline RMR ~28 kcal·kg LBM^{-1}·day^{-1}; Month 12 RMR ~33kcal·kg LBM^{-1}·day^{-1} 2.8 kg increase in BM Mense resumed after 23 days
Guebels et al., 2014	n= 8 females with amenorrhea n= 8 eumenorrheic females Engaging in >7 hrs/wk of exercise	Metabolic cart (Pavo) with hood Overnight fasted with 11-24 hrs since last exercise 20-30 min rest, 8-10 min measurement with steady state to calculate RMR Body composition via DXA	360 kcal/day increase in EI for 6 months	No effect of intervention on RMR 1.6 kg increase in BM All participants restored menses
Flueck et al., 2022	n= 1 wheelchair badminton player with suppressed RMR ratio	Metabolic cart (Oxycon Pro) Overnight fasted No information on gas collection device, exercise restriction, rest period or length of measurement Body composition via DXA	200-400 kcal/day increase in EI In addition to normal training, 2 additional arm crank exercise sessions per week	RMR ratio >1.0 at annual follow-up visit 11.8 kg decrease in BM with 11 kg of this FM loss

Body mass, BM; DXA, dual-energy X-ray absorptiometry; EA, energy availability; EI, Energy intake; FM, fat mass; LEA, low energy availability; LBM, lean body mass; RMR, resting metabolic rate; TEE, total energy expenditure.

2.1.1.3 RMR in response to a LEA intervention

The first study to examine the effect of a LEA intervention on RMR in athletic cohorts did not occur until 2020 (Kojima et al., 2020) with 6 additional studies being published since then (see Table 2.2). The majority of these studies are of acute duration with LEA interventions ranging from 3-14 days, although 1 case-study implemented an 8-week LEA intervention (Langan-Evans et al., 2021). Of these 7 studies, three reported a decrease in RMR following a period of LEA. This included a reduction in the RMR of a male combat athlete by -257 kcal·day^{-1} following 7 weeks of EA ~20 kcal·kg FFM^{-1}·day^{-1} and then 1 week of EA ~3 kcal·kg FFM^{-1}·day^{-1} (Langan-Evans et al., 2021), a reduction in the RMR of recreationally active females by -65 kcal·day^{-1} following 10 days of EA ~25 kcal·kg FFM^{-1}·day^{-1} (Oxfeldt et al., 2024), and a reduction in the RMR of sedentary females following just 3 days of ~15 kcal·kg FFM^{-1}·day^{-1} (Hutson et al., 2024). Contrary to these findings, interventions of similar duration (3-14 days) and magnitude of LEA (~15-22 kcal·kg FFM^{-1}·day^{-1}) had no effect on the RMR of male athletes (Jurov, Keay, & Rauter, 2022; Kojima et al., 2020; Sim et al., 2024) and one study involving female athletes (Caldwell et al., 2024). These conflicting findings may be due to methodological differences or changes in RMR in response to LEA may be impacted by moderating factors, such as sex, dietary characteristics and/or training characteristics.

Table 2.2. Studies that have examined the impact of a LEA intervention on RMR.

Reference	Population	RMR Protocol	Design & Intervention	Results
Kojma et al., 2020	n=7 male long-distance runners	Metabolic cart (AE310S) 10 min measurement with last 3 min used Body composition via BIA (InBody770) No info on gas collection device, exercise or fasting requirements, rest period or gas exchange data selection method	Randomised crossover with 3 days of: 1. LEA ~19 kcal·kg FFM^{-1}·day^{-1} 2. HEA ~53 kcal·kg FFM^{-1}·day^{-1} 75 min/day of running at 70% of VO_2	No effect of EA on RMR 1 kg decrease in FFM with LEA
Langan-Evans et al., 2021	n= 1 male combat athlete	Metabolic cart (GEM Open Circuit) >8 hrs fast with >10 hrs no exercise 25 min measurement with last 10 min used Body composition via DXA No info on gas collection device used or gas exchange data selection method	8 wk case study of EA ~20 kcal·kg FFM^{-1}·day^{-1} for 7 wks followed by EA ~3 kcal·kg FFM^{-1}·day^{-1} for 1 wk	257 kcal/day decrease in RMR 3.6 kg decrease in FFM
Jurov et al., 2022	n=12 male endurance athletes	Metabolic cart (K5) with facemask 12 hrs fast and >12 hrs no exercise 30 min measurement with last 20 min used Body composition via BIA (InBody770) No info on rest period or gas exchange data selection method	Pre-post 2 wks of EA ~22 kcal·kg FFM^{-1}·day^{-1} Achieved by increasing EEE without changing reported EI	No change in absolute RMR Decreased FFM
Oxfeldt et al., 2024	n= 30 recreationally active females	Metabolic cart (Q-NRG) with hood Overnight fasted 5 min rest period 20 min measurement with last 15 min used	Parallel group design with both groups starting with 5 days of EA ~50 kcal·kg FFM^{-1}·day^{-1} followed by 10 days of: 1. LEA ~25 kcal·kg FFM^{-1}·day^{-1} 2. HEA ~50 kcal·kg FFM^{-1}·day^{-1}	65 kcal/day decrease in absolute RMR with LEA 0.4 kg decrease in FFM with LEA and 0.4 kg increase in FFM with HEA

		Body composition via DXA No info on exercise restriction, or gas exchange data selection method	4 day training block with resistance exercise and moderate to high-intensity exercise on bicycle ergometer	
Sim et al., 2024	n= 12 male distance runners	Metabolic cart (Parvo) with hood Overnight fasted and >18 hrs no exercise 30 min measurement with average of last 15 min used to calculate RMR Body composition via BIA (InBody770) No info on rest period	Randomised crossover with 4 days of: 1. HEA ~45 kcal·kg FFM^{-1}·day^{-1} 2. LEA ~30 kcal·kg FFM^{-1}·day^{-1} 3. LEA ~15 kcal·kg FFM^{-1}·day^{-1} Daily EEE of kcal·kg FFM^{-1}·day^{-1} by running at 65% of VO_2 max	No change in absolute RMR or FFM across conditions
Hutson et al., 2024	n=19 sedentary females	Douglas bag with mouthpiece Overnight fasted 15 min rest and 5 min familiarisation with mouthpiece 5 min x 2 Douglas bags Body composition not assessed and no info on exercise restriction	Randomised crossover with 3 days of: 1. LEA ~15 kcal·kg FFM^{-1}·day^{-1} 2. HEA ~45 kcal·kg FFM^{-1}·day^{-1} No exercise throughout	Decrease in absolute RMR with LEA
Caldwell et al., 2024	n= 12 endurance trained females	Metabolic cart (Vyntus CPX) Overnight fasted 25 min measurement with lowest energy expenditure during last 20 min used to calculate RMR Body composition via DXA No info on gas collection device used or exercise restriction	Randomised crossover with 14 days of: 1. HEA ~52 kcal·kg FFM^{-1}·day^{-1} 2. LEA ~22 kcal·kg FFM^{-1}·day^{-1} Maintained normal exercise routine (~8 hr/wk of exercise)	RMR decreased with both LEA (-166 kcal/day) and HEA (-210 kcal/day) Decreased FFM (-0.8 kg) and FM (-1.2 kg) with LEA only

DXA, dual-energy X-ray absorptiometry; EEE, exercise energy expenditure; EI, energy intake; FM, fat mass; FFM, fat free mass; HEA, high energy availability; LEA, low energy availability; RMR, resting metabolic rate; VO_2 max, maximal oxygen consumption.

2.1.2 Body composition and stature

Although discrete sites of energy production can be measured at the cellular level *in vitro,* there are difficulties in integrating this information to the *in vivo* model (Wang et al., 2000). Therefore, the first practical level to consider metabolic rate differences occurs at the tissue/organ level, where the variability is exemplified by the estimated metabolic rate of ~440 $kcal \cdot kg^{-1} \cdot day^{-1}$ for the kidney compared to estimations of ~13 $kcal \cdot kg^{-1} \cdot day^{-1}$ for skeletal muscle (Wang et al., 2010). In terms of overall contribution to the metabolic rate of the whole body, the individual mass of tissue/organs needs to be considered in addition to their metabolic rate (Wang et al., 2000). For instance, although skeletal muscle and adipose tissue are the largest component of BM, their metabolic rate is low compared to more metabolically active organs like the liver, kidneys, heart and brain which only account for 5-6% of BM (Wang et al., 2000). At the whole-body level, although there is a long-recognised correlation between BM and metabolic rate (Kleiber, 1947), BM alone only explains ~50% of the variance in RMR (Müller et al., 2018). Rather, FFM is the major determinant of RMR and explains ~60-85% of variance in RMR (Cunningham, 1991; Johnstone et al., 2005; Müller et al., 2018; Oshima et al., 2012). Because of this, RMR is often expressed relative to FFM and a value <30 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ has been suggested as an indicator of LEA (Stellingwerff et al., 2023). However, this threshold does not consider that the relative proportion of metabolically active components changes as FFM increases (Weinsier et al., 1992). As FFM increases, a decreased proportion of this FFM is made up of high metabolic rate organs (i.e. liver, kidney, heart) resulting in a lower relative RMR (Heymsfield et al., 2002; Wang et al., 2000). A person's stature must also be considered due to differences in proportions of organs and tissues with differing heights (Heymsfield et al., 2021). Any organ that scales to height with a power of <2 will be present as a smaller fraction of BM in those who are tall whereas organs scaling to height with powers >2 will comprise a larger fraction of BM (Heymsfield et al., 2019). For example, increases in

height are associated with a decrease in the proportion of brain mass to BM and an increase in the proportion of skeletal mass to BM resulting in a lower relative RMR in those taller compared to those shorter in stature (Heymsfield et al., 2019). This is an important consideration in sport where athletes can exhibit high levels of absolute FFM with extreme differences in morphology both compared to the general population and also differences between different athletic populations (e.g. a female gymnast vs male shot putter) that may change the proportion of tissues/organs that contribute to overall metabolic rate. Therefore, body composition and stature have implications for overall RMR, and the relative RMR threshold that is used as an indicator of LEA. Indexing RMR to regional estimates of FFM (i.e. trunk or limb FFM) may improve its use as more metabolically active tissue are located in the trunk. Additionally, creating a normative database for RMR in athletic cohorts may provide insight on how the RMR to FFM relationship changes with body composition and stature.

2.1.3 Sex

Men typically have a higher absolute RMR than women as a result of their larger BM and FFM (Wells, 2007), with sex differences in absolute RMR typically disappearing when adjusted for differences in FFM in both non-athletic (Ferraro et al., 1992; Johnstone et al., 2005; Klausen et al., 1997; Perseghin et al., 2001) and athletic cohorts (Jagim et al., 2019, 2023; Thompson & Manore, 1996). However, this is not a consistent finding as others have reported sex differences in RMR, even when differences in body composition are accounted for (Arciero et al., 1993). Here, the failure to detect sex-differences in RMR may be due to the use of a two-compartment body composition model which fails to distinguish the heterogeneity of the components of LBM in relation to metabolic rate (Moore, 1980). In support of this, sex differences in RMR were observed when adjusted for body cell mass, but not when adjusted for LBM (Buchholz et al., 2001). While the evidence for sex-differences in RMR in adult populations are conflicting, studies of RMR in children of 6-17 years (Garn & Clark, 1953;

Kaneko et al., 2013; Kirkby et al., 2004; Molnár & Schutz, 1997), and adults >65 years (Fukagawa et al., 1990; Poehlman et al., 1997) show consistent results, with male populations having a greater RMR than female populations. Notably, the RMR ratio thresholds that are used to indicate a suppressed RMR were validated in female populations (Strock et al., 2020), and different thresholds may be appropriate for male populations. Furthermore, because the FFM of women is made up of a larger fraction of high metabolic rate organs and tissues than men, they tend to have a greater relative RMR (Geer & Shen, 2009). As such, the relative RMR and RMR ratio thresholds indicative of LEA may differ between male and female athletes.

2.1.4 Race

Racial differences in RMR have been reported with most studies comparing Caucasian and African American populations. These studies consistently show a lower relative RMR in African Americans compared to Caucasians (Adzika Nsatimba et al., 2016; Albu et al., 1997; Carpenter et al., 1998; Gannon et al., 2000; Kushner et al., 1995; Martin et al., 2004; Sharp et al., 2002; Spaeth et al., 2015). However, differences disappear after adjusting for trunk LBM, but not when adjusting for limb LBM (Hunter et al., 2000). As more metabolically active organs are in the trunk, this suggests that a lower organ mass is responsible for the lower RMR observed in African Americans (Hunter et al., 2000). This has been supported by MRI imaging studies which demonstrate a higher fraction of FFM as skeletal mass and lower fraction of FFM as high metabolic rate organs in African American compared to Caucasian populations (Gallagher et al., 2006; Jones et al., 2004). Other racial differences in RMR have been less studied but include findings of a lower RMR in Polynesian (Rush et al., 1997), Indian (Mason et al., 1964), and south Asian (Bakker et al., 2014) populations compared to Caucasian populations. Yet, this is not a consistent finding as others have reported no difference in the RMR between Caucasian and Asian populations (Soares et al., 1998; Wouters-Adriaens & Westerterp, 2008). A study comparing Chinese, Asian-Indian and Malaysians populations

reported a lower RMR in Asian-Indians than Chinese, but after adjusting for differences in trunk FFM, the differences were no longer evident, suggesting that differences were due to different proportions of metabolically active organs (Song et al., 2016). Notably, most RMR predictive equations have been developed with Caucasian participants and may not accurately predict RMR in non-Caucasian populations (Reneau et al., 2019). If these predictive equations overestimate or underestimate RMR in non-Caucasian populations, then this would increase the risk of a false or positive diagnosis of LEA if using a RMR ratio. Evidently, an athlete's race is another factor that must be considered when using RMR measurements as an indicator of LEA.

2.1.5 Age

RMR changes across the lifespan, starting with variations in the first year of life due to changes in body composition at different stages of infancy (FAO/WHO/UNU, 2004). While children have a lower RMR than adults due to their smaller BM, they have a higher ratio of RMR relative to BM (Wang, 2012). For instance, the adjusted RMR of a 9–15-month infant is elevated by ~50% compared with that of an adult (Pontzer et al., 2021). This higher ratio in children is attributed to a higher proportion of BM being highly metabolic tissue and/or higher metabolic rate of organs and tissues (Holliday, 1971; Hsu et al., 2003; Müller et al., 2018; Wang, 2012; Weinsier et al., 1992). Throughout adulthood, there is a continued decrease in adjusted RMR with a break point around ~20 years of age where it remains stable to around ~60 years of age and then continues to decrease (Pontzer et al., 2021). However, the 'break point' at which RMR decreases with age may differ between sexes, with this decline occurring earlier in women compared to men (Geisler et al., 2016; Siervo et al., 2015). Numerous studies have demonstrated that this decrease in RMR with age cannot be fully accounted for by the progressive loss of FFM that also occurs with age (Alfonzo-González et al., 2006; Frisard et al., 2007; Fukagawa et al., 1990; Gallagher et al., 2000; Geisler et al., 2016; Hunter et al., 2001;

Johnstone et al., 2005; Klausen et al., 1997; Krems et al., 2005; Lührmann et al., 2009; Piers et al., 1998; Poehlman et al., 1991, 1993; Roberts et al., 1995; Vaughan et al., 1991; Visser et al., 1995; Wang et al., 2005). Rather, this decline in RMR may be due to a lower RMR per unit cell mass of individual organs and tissues and/or the cellular fraction of organs and tissues may differ with age (Bosy-Westphal et al., 2003; Gallagher et al., 2000; Müller & Bosy-Westphal, 2013; Wang et al., 2005, 2010). Notably, habitual physical activity may prevent the decline in RMR that occurs with the age-independent of changes in FFM (Poehlman et al., 1990, 1991; Van Pelt et al., 1997). The change in RMR associated with ageing has implications for the relative RMR threshold that is used to indicate LEA, as this may not be appropriate for either youth or masters' athletes. Additionally, care should be taken when measuring RMR longitudinally in athletes <20 years or >60 years of age as an indicator of LEA, noting the decrease in RMR relative to FFM that occurs with ageing during these times.

2.1.6 Menstrual cycle phase

In eumenorrheic woman, cyclical changes in the primary sex hormones, estrogen and progesterone, occur throughout the various phases of the menstrual cycle (MC) (see Figure 2.2) (Elliott-Sale et al., 2021). There is evidence that sex hormones modulate RMR. For instance, the pharmacological suppression of sex hormones via GnRH antagonist therapy in pre-menopausal women reduces RMR (Day et al., 2005). Later, it was shown that this reduction in RMR with GnRH antagonist therapy can be prevented by estrogen administration (Melanson et al., 2015). A meta-analysis published in 2020 showed a small effect favouring an increase in RMR during the luteal phase of the MC compared to the follicular phase (Benton et al., 2020). The role of estrogen in regulating RMR is supported by higher estrogen concentrations during the luteal phase of the MC compared to the early to mid-follicular phase. Although progesterone is also high in the luteal phase of the MC, and plays a role in the increased body temperature that is seen near ovulation and throughout the luteal phase of the MC (Nakayama

et al., 1975), it is not thought to play a major role in regulating RMR (Metz et al., 2022). This is supported by evidence that the increase in sleeping metabolic rate (SMR) seen during the luteal phase of the MC cannot fully be explained by the rise in body temperature (Zhang et al., 2020). Most studies measuring RMR across the MC compare Phase 1, when both estrogen and progesterone are low, to Phase 4, when both estrogen and progesterone are high (see. Elliott-Sale et al., 2021 for phase definitions). However, Phase 2, when estrogen levels are at their highest and progesterone levels remain low, and Phase 3, when there are medium levels of estrogen (higher than Phase 1 but lower than Phase 2 and 4) and progesterone levels still remain low (higher than Phase 1 but <6.4 $nmol \cdot L^{-1}$) (see Elliott-Sale et al., 2021 for phase definitions), present unique hormonal profiles that would allow insight into the separate roles of estrogen and progesterone on RMR. Two studies have assessed RMR in these phases and while Best Practice Guidelines were not followed for control of ovarian hormones (Elliott-Sale et al., 2021), no difference in RMR was found in Phase 2 (Howe et al., 1993) or in Phase 3 (Paolisso et al., 2000) compared to Phase 1 and Phase 4 of the MC. Despite the call for more quality research in the 2020 meta-analysis, (Benton et al., 2020), there are only three subsequent studies of changes in RMR across the MC of healthy women. One study failed to find differences in RMR between the early follicular and mid-follicular phase of the MC (Gould et al., 2021). The remaining two studies compared RMR in the follicular and luteal phase of the MC (Löfberg et al., 2024; Maury-Sintjago et al., 2022). However, despite one finding an increased RMR in the luteal phase, the failure to confirm MC phase by measuring ovarian hormones or detect for ovulation means that results should be interpreted with caution (Maury-Sintjago et al., 2022). The other study reported that RMR remained unchanged from the follicular to mid-luteal phase of the MC in recreationally active females, but when outliers (n=4) were removed, a 44 $kcal \cdot day^{-1}$ (~3%) increase in RMR was observed in the mid-luteal phase (Löfberg et al., 2024). Evidently, there is a lack of high-quality research examining if

meaningful changes in RMR occur across the different hormonal profiles of the MC in athletic cohorts. Yet, this has implications for the standardisation of MC phase when measuring RMR in athletic cohorts and/or when interpreting RMR measurements longitudinally in female athletes who may have measurements at different phases of the MC.

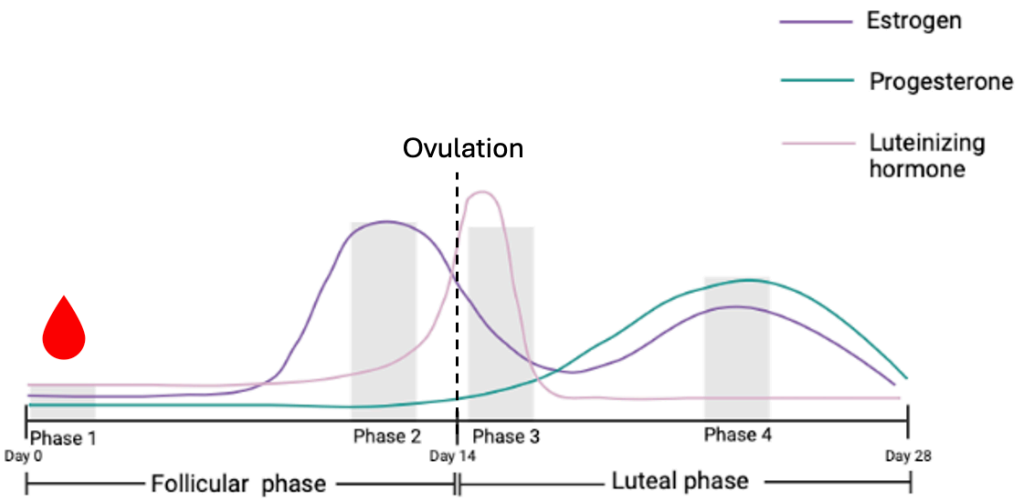

Figure 2.2. Overview of the fluctuations of estrogen, progesterone and luteinising hormone across a typical 28-day ovulatory menstrual cycle. Day 1 indicates the onset of menstruation with ovulation occurring on day 14.

2.1.7 Hormonal contraceptive usage

Given the common usage of hormonal contraceptive (HC) among female athletes (Martin et al., 2018; McNamara et al., 2022; Oxfeldt et al., 2020) and their effect on endogenous sex hormones synchronous exogenous profiles (Elliott et al., 2005; Hirschberg, 2022), the effect of HC use on RMR must be considered. Most studies examining the impact of HC usage on RMR have examined the oral contraceptive pill (OCP), which down-regulate endogenous ovarian hormones when compared to the luteal phase of the MC and provide synthetic ovarian hormones (Elliott et al., 2005). There are various regimens, but standard regimens involve taking a daily pill containing a combination of both estrogen and progesterone or progestin-only for 21 days, followed by a 7-day pill free interval (Christin-Maitre, 2013). Studies

examining the effect of OCP usage on RMR are complicated by the numerous types and formulations of available OCPs, with different effects on endogenous ovarian hormones and variability in exogenous ovarian hormone concentrations (Elliott-Sale et al., 2013). Most studies are cross-sectional in nature, comparing the RMR of OCP users to naturally cycling (NC) women, with few implementing Best Practice Guidelines for the control of ovarian hormones (Elliott-Sale et al., 2021). The majority of studies have failed to detect a difference in the absolute RMR between OCP users and non-users (Duhita et al., 2017, 2019; Eck et al., 1997; Jensen & Levine, 1998), although one study reported a higher RMR in OCP users when FFM and FM were included as covariates (Diffey et al., 1997). Contrary to this finding, the single locatable investigation of RMR before and after OCP usage found a decrease in absolute RMR with OCP usage in the absence of a change in BM (McNeil et al., 1988). While these results provide rare insight on how exogenous hormones affect RMR, they have limited applicability today as the specific OCP formulation and dose (30 $\mu g \cdot day^{-1}$ Levonorgestrel) is typically no longer prescribed (McNeil et al., 1988). In addition to comparing differences between OCP and non-OCP users, differences across OCP phases must be considered (i.e. active vs. inactive days) in view of the variation in concentrations of endogenous and exogenous hormones across these days (Elliott-Sale et al., 2021). However, only one study investigating differences in RMR across the OCP cycle could be located. This study showed no difference in RMR between the active phase and hormone free interval of monophasic combined OCP users (Löfberg et al., 2024). Another form of HC used by women includes the injection of depot-medroxyprogesterone acetate (DMPA) every 3 months, which works by suppressing the hypothalamic-pituitary-ovarian axis leading to reduced estrogen and luteinising hormone (LH) levels (Steward et al., 2016). While one study reported an increase in absolute RMR following 12 months of DMPA usage, participants also had a significant increase in BM (Batista et al., 2017). When changes in RMR were compared between

participants who experienced >3 kg of BM gain and those that gained <3 kg, increases in RMR were only significant for the group with the larger BM gains (Batista et al., 2017), suggesting that the increase in RMR was secondary to increases in BM rather than DMPA. Other studies examining the effect of DMPA on RMR have found no effect on RMR (Pelkman et al., 2001; Steward et al., 2016). When examined in peri-menopausal women, the use of progestin only and intra-uterine systems for 12 months did not result in a significant change in absolute RMR despite the observed increase in body fat (Napolitano et al., 2016). No studies of other HCs (e.g. contraceptive patches or vaginal rings) on RMR in pre-menopausal women could be found.

2.1.8 Training status

An athlete's training status or load may contribute to variability in RMR measurements due to the associated changes in body composition and/or the adaptations to physiology that occur with chronic training. For instance, the increase in beta-adrenergic stimulation with exercise training may increase RMR (Tremblay, Coveney, et al., 1992; Zouhal et al., 2008). As an athlete's training status will likely vary across a training cycle with changes in training load, it is important to distinguish the effects of training status on RMR. Given the high pertinence of this factor, literature reviewed will be broken down into studies measuring RMR 1) in athletes versus non-athletes 2) following exercise cessation and 3) with variations in training load.

2.1.8.1 Athletes versus non athletes

Cross-sectional studies comparing the RMR of athletic and non-athletic populations give conflicting results with findings of both no difference (Bowden & McMurray, 2000; Broeder et al., 1992; Byrne & Wilmore, 2001; Hill et al., 1984; Poehlman et al., 1985, 1991; Ratcliff et al., 2011) as well as a higher RMR in athletic populations compared to sedentary populations even after adjusting for differences in FFM (Gilliat-Wimberly et al., 2001; Poehlman et al., 1988; Sjodin et al., 1996; Toth et al., 1995). Conflicting results may be due to differing calibre

of athletes across studies. For instance, in one study that reported a higher RMR in athletes compared to sedentary controls, the athletes were male members of a varsity cross-country team of Tier 3 calibre (Highly Trained/National level) (McKay et al., 2022), training 100-160 $km \cdot wk^{-1}$ with a maximal aerobic capacity (VO_2 max) of 71 $ml \cdot kg^{-1} \cdot min^{-1}$ (Poehlman et al., 1988). In contrast, another study found no difference in RMR between sedentary controls and male runners of Tier 2 calibre (Trained/Developmental) (McKay et al., 2022) with a weekly mileage of 45 $km \cdot wk^{-1}$ and a VO_2 max of 57 $ml \cdot kg^{-1} \cdot min^{-1}$ (Toth et al., 1995). Contrasting findings may be explained by differences in the physiological adaptations achieved by the training volume. An alternative explanation is that the failure to restrict exercise the day prior to a RMR measurement in athletic cohorts resulted in a falsely elevated RMR due to residual excess post-exercise oxygen consumption (EPOC). However, in the study where differences in RMR were reported between athletic and non-athletic cohorts, exercise was restricted for >24 hours prior to the RMR measurement (Poehlman et al., 1988). Nevertheless, Residual EPOC leading to erroneous RMR measurements will be discussed in section 2.3.3.

2.1.8.2 Exercise cessation

The majority of studies report a reduction in RMR following exercise cessation. This includes a ~7-8% decrease in RMR following 3 days of exercise cessation in long-distance runners (Herring et al., 1991; Tremblay et al., 1988) and a ~4% decrease in RMR following 7-10 days of exercise cessation in endurance trained rowers, cyclists, and swimmers in the absence of change in BM or skinfold thickness over the same period (Arciero et al., 1998). Similar results have been seen with more chronic periods of exercise cessation such that a ~7% decrease in absolute RMR was reported following 35-42 days of detraining in female division III swimmers with an increase in FM and no change in LBM being observed (Ormsbee & Arciero, 2012). It is important to note that these findings may also be confounded by a falsely elevated RMR measurement from residual EPOC during the training phase, since exercise was not

controlled during the 24 hours prior to the first RMR measurement in these studies. Interestingly, the only study located which failed to observe a decrease in RMR with 3 weeks of detraining in a cohort of male athletes engaged in various sports, implemented a 36-hour exercise restriction prior to the first RMR measurement (LaForgia et al., 1999). This suggests that prior reports of decreases in RMR with exercise cessation were an artefact of a persistent EPOC which falsely elevated RMR measurements prior to exercise cessation (LaForgia et al., 1999).

2.1.8.3 Variations in training load

Conflicting results are seen when measuring RMR across a training season or with variations in training load in athletic cohorts (Drenowatz et al., 2013; MacKenzie-Shalders et al., 2019; Nishisaka et al., 2022; Zabriskie et al., 2019). Notably, increases in FFM that occur with training variations across a season are not always accompanied by increases in RMR (MacKenzie-Shalders et al., 2019; Westerterp et al., 1994). For instance, in one study of rugby union players, an increase in LBM by 2.0 kg over a 14-week preseason was not accompanied by a change in RMR (MacKenzie-Shalders et al., 2019). The lack of absolute change in RMR may be explained by insufficient precision in RMR measurement to detect a theoretical increase of ~26 $kcal \cdot day^{-1}$ associated with 2.0 kg increase in skeletal muscle mass (Wang et al., 2010). It is also possible that concurrent LEA with variations in training load was confounding results and contributing to the conflicting findings.

Alternatively, in non-athletic populations, an energy compensation model has been proposed in which increases in EEE may be counterbalanced by a reduction in energy expenditure from other sources, including a decrease in RMR (Careau et al., 2021). Decreases in RMR with increased training volume is further supported by a 2022 meta-analysis finding a decrease in RMR in overreached athletes following a period of intensified training (Kuikman et al., 2022)

with overreaching being characterised by reduced performance following a period of increased training load (Halson & Jeukendrup, 2004). It is possible that the reduced RMR with overreaching is due to LEA, following a failure to increase EI with increases in training load. However, it is also possible that high levels of training stress, independent of energy status, contribute to a suppressed RMR. Mechanistically, this could occur due to underlying changes in autonomic nervous system activity that have been shown to occur with overreaching (Coates et al., 2018; Flatt et al., 2017; Le Meur et al., 2013). Evidently, more research is needed examining the impact of training status on RMR while controlling for EI to elucidate the separate effects of training load and LEA on RMR.

2.1.9 Energy flux

Energy flux, representing the combination of EEE and EI under conditions of energy balance, is related to training load. A high EEE with matched levels of EI results in high energy flux, whereas low EEE with matched levels of EI results in low energy flux (Melby et al., 2019). Energy flux is hypothesised to be a determinant of RMR, via sympathetic support (Bell et al., 2004). Consistent with this, RMR was increased 3% above baseline following 10 days of increasing both EEE and EI by 1100-1300 kcal·day^{-1}, creating a state of increased energy flux in men (Goran et al., 1994). Shorter periods of energy flux manipulation have also influenced RMR, with a greater RMR seen after three days of high energy flux (EI ~4400 kcal·day^{-1}, EEE ~1500 kcal·day^{-1}) compared to three days of low energy flux (EI ~2600 kcal·day^{-1}, EEE ~0 kcal·day^{-1}) in trained men (Bullough et al., 1995). A state of increased energy flux has been proposed to help with weight loss maintenance, with a greater RMR apparent after four days of high energy flux (EI ~3200 kcal·day^{-1}, EEE ~500 kcal·day^{-1}) compared to low energy flux (EI ~2500 kcal·day^{-1}, EEE ~0 kcal·day^{-1}) that was preceded by a 7% loss of BM over an 8-12 week period in males and females with obesity (Paris et al., 2016). These studies suggest that RMR may be increased in athletes under conditions of an increased training load and matched

increase in EI. However, it is possible that these findings of increased RMR under high energy flux versus low energy flux conditions were an artifact of residual EPOC and TEF due to the increased EEE and EI required to achieve a state of high energy flux.

2.1.10 Circadian phase

The time at which RMR measurements occur must also be considered. Many studies examining circadian changes in RMR are confounded by the effects of prior EI or activities of daily living (Acosta et al., 2021; Haugen et al., 2003; Miles et al., 1993; Rynders et al., 2020; Weststrate et al., 1989). Two studies have measured RMR periodically using indirect calorimetry during extended periods of fasting from 0900 to 1700h (Zurlo et al., 1986) and 0800 to 1600h (Leff et al., 1987), with both finding no difference in RMR during these time periods (Leff et al., 1987; Zurlo et al., 1986). However, these studies are limited to an 8-hour duration. By shifting the sleep-wake cycle to 28 hours (16.33 hours wake, 11.67 hours sleep) for 21 days, circadian changes independent of food intake and behavioural state could by examined over a 24-hour period (Zitting et al., 2018). Using this protocol, RMR was lowest in the late biological night (0500h) and peaked 12 hours later with a difference of 129 kcal·day^{-1} between the peak and trough (Zitting et al., 2018). While this suggests that the time at which RMR measurements occur may create variability in the measurement, RMR measurements are likely to occur within a short period in the morning due to the requirement that athletes are overnight fasted and rested. A consistent and narrow time window should be used for testing to account for potential circadian changes in RMR, and the time at which RMR measurements occur should be noted.

2.2 Technical and environmental sources of variability

The following section will highlight technical and environmental sources of variability in RMR measurements (see Figure 2.3). These relate to the practices and procedures used when measuring RMR that may contribute to variability between test visits or between different testing environments. Each source of variability will again be discussed in sub-sections below.

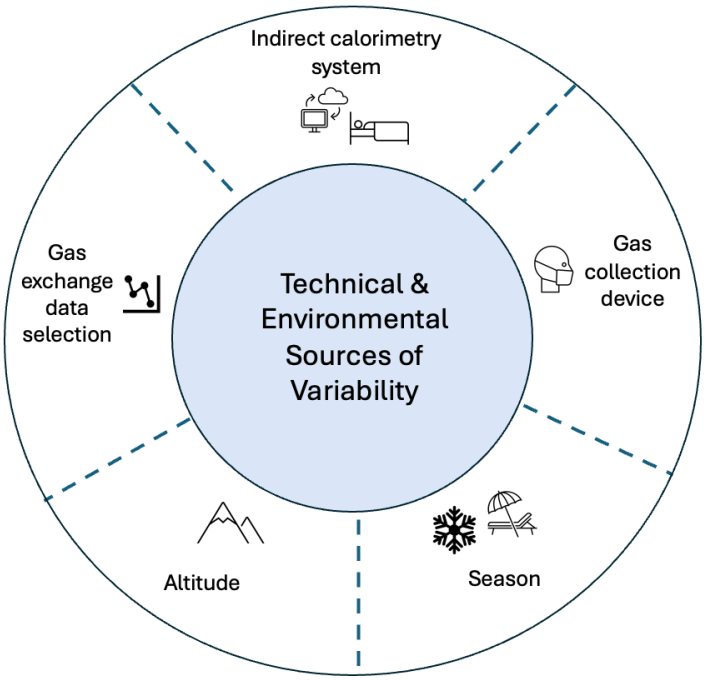

Figure 2.3. Potential technical and environmental sources of variability in RMR measurements.

2.2.1 Indirect calorimetry system

Methods of indirect calorimetry include the Douglas Bag method, metabolic cart, and whole room calorimetry (Haugen et al., 2007). Most studies have reported inter-machine differences in measured RMR when using a metabolic cart (Alcantara et al., 2018, 2022; Kennedy et al., 2014) as well as differences between RMR measured by a metabolic cart compared to the Douglas bag method (Woods et al., 2016) and whole room calorimetry (Chen et al., 2020; Rising et al., 2015). As such, athletes should have repeat measurements of RMR with the same indirect calorimetry system. There are more novel methods of indirect calorimetry available on the market, such as handheld devices and portable metabolic carts. While not a consistent finding (Stewart et al., 2005), compared to metabolic carts, RMR measurements are greater with handheld devices (Alam et al., 2005; Frankenfield & Coleman, 2013; Melanson et al., 2004). Differences in RMR measured between portable calorimetry and metabolic carts (Leal-

Martín et al., 2023; Littlewood et al., 2002; Wahrlich et al., 2006; Yeung et al., 2020) as well as whole room calorimetry (Purcell et al., 2020) have also been reported, but this too is not a consistent finding (Welch et al., 2015). Clinical and research uses of RMR assessments obtained from handheld devices and portable metabolic carts should be undertake with caution unless the equipment and methods have been validated.

The inter and intra-day reliability of an individual indirect calorimetry system should also be considered. Numerous studies have assessed the inter (see Table 2.3) and intra-day reliability (see Table 2.4) in RMR measurements using a metabolic cart with variable results. However, the standardisation methods used by these studies (i.e. period of rest, familiarisation, technician error etc) may contribute to the inter- and intra-day reliability reported. For instance, day-to-day variability may change with the gas collection device used (Roffey et al., 2006) or method of gas exchange data selection used to calculate RMR (see section 2.2.3) (Alcantara et al., 2023). Fewer studies have assessed inter- or intra-day reliability in RMR measured via Douglas bag method or whole room calorimetry. The available investigations have reported a 4.3% coefficient of variation (CV) in intra-day and 6.6% CV in inter-day reliability when using the Douglas bag method (Woods et al., 2016) and 2.7% CV in inter-day reliability when using whole room calorimetry (Henriksen et al., 2023). Best practice in both clinical and research uses of RMR measurements should include an understanding of inter- and intra-day machine reliability of their indirect calorimetry system in order to determine what is considered a meaningful change in longitudinal measurements of RMR.

Table 2.3. Inter-day reliability in measured RMR with varying metabolic carts.

Reference	Participants	RMR protocol	Time between measurements	Metabolic cart brand & gas collection system	Inter-day difference
Alam et al., 2005	n=37F Unknown calibre	10-12 hr fast 25 min rest 10 min familiarisation 10 min measurement	2.5 wks	Delatrac II with hood	4.5% CV
Roffey et al., 2006	n= 5M/5F Sedentary	10 hr fast 30 min measurement with Deltatrac 15 min measurement with MOXUS modular Listened to radio during measurement to prevent sleeping	>1 day	Deltatrac II with hood MOXUS Modular VO2 with mouthpiece	2.3% CV 3.1% CV
Blond et al., 2011	n=15M/15F Unknown calibre	Overnight fast 30 min rest 45 min measurement	24 hrs	Deltatrac II with canopy Quark RMR with canopy	26±93 kcal -20±86 kcal
Kennedy et al., 2014	n= 4M/16F Unknown calibre	10-12 hr fast 30 min rest 30 min measurement	2 wks	Deltatrac II with hood GEM with hood ECAL with mouthpiece	5.4% CV 6.9% CV 13.1% CV
Welch et al., 2015	n=28M/13F Unknown calibre	12 hr fast 10 min rest Ended when 10 min steady state achieved	24 hrs	Parvo TrueOne with mouthpiece	7.2±152 kcal

Woods et al., 2016	n=10M/10F Endurance athletes	Inpatient measurement 10 min familiarisation 20 min measurement	24 hrs	Parvo TrueOne with mouthpiece	6.3% CV
Alcantara et al., 2018	n=6M/11F Sedentary	8 hr fast 20 min rest 30 min measurement	24 hrs	CCM Express with face mask Ultima cardio 2 with face mask	13.5% CV 18.3% CV
Mackay et al., 2019	n=12F Recreationally active	10 hr fast 5 min rest 20 min measurement	1-5 days	Parvo TrueOne with hood	5.3% CV
Alcantara et al., 2022	n=18M/11F Unknown calibre	12 hr fast 20 min rest 30 min measurement	24 hrs	Q-NRG with canopy Omnical with canopy Vyntus with canopy Ultima with face tent	3.6% CV 4.8% CV 4.6% CV 5.7% CV
Iraki et al., 2023	n= 14M/12F Unknown calibre	Overnight fast 5 min rest 25 min measurement with first 5 min discarded	24 hrs	Vyntus CPX with canopy	4.5% CV

CV, coefficient of variation; F, female; M, male; RMR, resting metabolic rate.

Table 2.4. Intra-day reliability in measured RMR with varying metabolic carts.

Reference	Participants	RMR protocol	Time between measurements	Metabolic cart brand & gas collection system	Intra-day difference
Alam et al., 2005	n=37F Unknown calibre	10-12 hr fast 25 min rest 10 min familiarisation 10 min measurement	15 min	Delatrac II with hood	3.0% CV
Welch et al., 2015	n=18M/13F Unknown calibre	12 hr fast 10 min rest Ended when 10 min steady state achieved	Consecutive	Parvo TrueOne with mouthpiece	-19.4±83.4 kcal
Blond et al., 2011	n=15M/15F Unknown calibre	Overnight fast 30 min rest 45 min measurement	45 min	Deltatrac II with canopy Quark RMR with canopy	27±213 kcal 3±195 kcal
Woods et al., 2016	n=10M/10F Tier 2 athletes	Inpatient measurement 10 min familiarisation 10 min measurement	Consecutive	Parvo TrueOne with mouthpiece	4.4% CV
Iraki et al., 2023	n= 14M/12F Unknown calibre	Overnight fast 5 min rest 25 min measurement with first 5 min discarded	20-25 min	Vyntus CPX with canopy	7.0% CV

CV, coefficient of variation; F, female; M, male; RMR, resting metabolic rate.

2.2.2 Gas collection device

Common gas collection devices used for RMR measurement include a hood/canopy, mouthpiece plus nose clip, or facemask. An early study reported no difference between RMR measured with the hood and facemask in a cohort of men and women (McAnena et al., 1986). Similarly, no differences were detected when RMR was measured with the hood, facemask, and mouthpiece plus nose clip in men and women, despite reported differences in subjective comfort between devices (Segal, 1987). Here, most participants preferred the hood (n=8), or facemask (n=7) compared to the mouthpiece plus nose clip (n=1) (Segal, 1987). However, the lack of difference in RMR measurements in this study may be due to the short duration of the gas collection, with the average of 2 x 5-minute measurements within a 20-minute period being used to determine RMR (Segal, 1987). On the other hand, a study in which gas collection occurred continuously for 15-20 minutes reported that RMR was ~5.9-7.5% higher when measured by the mouthpiece plus nose clip compared to the hood (Forse, 1993; Roffey et al., 2006) and increased by ~5.9% when measured with the facemask compared to the hood (Forse, 1993). This supports the theory that a longer collection period is needed to amplify the effect of participant discomfort on RMR outcomes, Finally, a study of men and women found RMR was ~150 $kcal \cdot day^{-1}$ higher when measured with facemask compared to hood in men, but not women, which was speculated to be due to differences in jaw shape or facial hair (Dupertuis et al., 2022).

It is notable that the study which validated the RMR ratio thresholds used the ventilated hood to measure RMR (Strock et al., 2020). Given the variability introduced by the gas collection device used, different thresholds may be needed when using a facemask or mouthpiece plus nose clip to measure and interpret RMR. The effect of a gas collective device on the ability to reach steady state (>5 minute period with ≤10% CV in VO_2 and VCO_2) must also be

considered, with steady state being easier to achieve with the ventilated hood system compared to the facemask (Wang et al., 2017), This has important implications for the gas exchange data selection method (see section 2.2.3). To our knowledge, no study has assessed the effect of mouthpiece plus nose clip on the ability to reach steady state during RMR measurements. As there is evidence that the gas collection device used for RMR measurements may introduce variability, repeat measurements should occur with the same gas collection device. Familiarity and/or discomfort with the gas collection device will be discussed in section 2.3.4 as a potential source of error in RMR measurements.

2.2.3 Gas exchange data selection method

When performing indirect calorimetry with a metabolic cart, gas exchange data is recorded for a pre-defined period (i.e. 30 minutes) and then a period within this window is used to calculate energy expenditure using the Weir equation (Weir, 1949). Common methods of gas exchange data selection include:

- Steady state: Time period with <10% CV in VO_2 and VCO_2
- Time interval approach: Values averaged over a pre-defined time interval (i.e. minutes 5-25 of measurement)
- Filtering method: Thresholds are set and depending on the mean RMR value obtained data is discarded (i.e. discarding values <85% or >115% of average RMR)

Current Best Practice Protocols recommend discarding the first 5 minutes of data and using >4 minutes of steady state data to calculate RMR, with steady state representing a period with <10% CV in VO_2 and VCO_2 (Fullmer et al., 2015). Although the evidence supporting this recommendation was graded as "weak" at the time of the protocol development a decade ago (Fullmer et al., 2015), subsequent studies have confirmed the consistency of steady-state data in producing a ~2-5% lower RMR value (Borges et al., 2019; Irving et al., 2017; Sanchez-Delgado et al., 2018). However, in some scenarios, participants may not achieve >4 minute of

steady state during the RMR test (Irving et al., 2017; Popp et al., 2016). Of the two studies which have compared methods of gas exchange data selection in athletic cohorts, one reported a ~2% lower RMR with the use of a high filter method (discarding value <95% or >105% of average RMR) when measured over 30 minutes (Freire et al., 2021). However, this study did not implement a rest or acclimatisation period prior to starting the measurement (Freire et al., 2021). The other reported a ~2.5% lower RMR when using 5 minutes of steady state data in a cohort of young Tier 3 athletes (12-18 years) (Bittencourt et al., 2023). As most studies continue to support the use of a steady state data to calculate RMR, this approach should continue to be used. Where there is a failure to achieve steady state during a RMR measurement, a repeat measurement may be warranted.

2.2.4 Altitude

Early studies reported increases in RMR with short term exposure (3-5 days) to high altitude (>3000 m) (Grover, 1963; Huang et al., 1984). Studies looking at longer periods of altitude exposure on RMR in men have reported a ~27% increase in RMR on day 2 of exposure to ~4300 m, with RMR then decreasing and plateauing to a ~17% increase in RMR above baseline by day 10 (Butterfield et al., 1992). Women showed a ~7% increase in RMR on day 3 of exposure to ~4300 m, with RMR then returning to sea-level values by day 6 (Mawson et al., 2000). Similar trends of initial increases in RMR with a return to baseline levels by 7 days of exposure to 4300 m in women have been reported by others (Hannon & Sudman, 1973). Endurance training status does not appear to alter the increase in RMR with acute exposure to high altitude; a similar increase in RMR (~12-13% above baseline) was reported in sedentary and endurance trained men after 34 hours of exposure to 3450 m (Sareban et al., 2020). Despite this evidence that RMR is increased at high altitudes, most research and clinical uses of RMR measurements in athletes are unlikely to be located at high altitude. On the other hand, there may be interest in measuring RMR at a low to moderate altitude (~1600-2400 m), since athletes

may periodise training camps of ~2-4 weeks at a low to moderate altitude into their training cycle (Stellingwerff et al., 2019). An early study reported a ~12% increase in RMR on day 5-6, and ~14% increase in RMR on day 10-11 of 1850 m altitude exposure in a cohort of men and women (Terzioglu & Aykut, 1954). Yet, there were no differences in RMR on day 2-3 of altitude exposure in this study (Terzioglu & Aykut, 1954). Only one study has assessed changes in RMR at a low to moderate altitude specifically in athletic cohorts, finding a ~19% increase in the RMR of male and female middle-distance runners at the end of a 4-week altitude training camp at ~2200 m (Woods, Sharma, et al., 2017). However, given the small sample size (3M/2F), this study is likely underpowered (Woods, Sharma, et al., 2017). Furthermore, it is possible that an even greater increase in RMR occurred with acute altitude exposure in this cohort of athletes, as has previously been seen at higher altitudes (Butterfield et al., 1992; Hannon & Sudman, 1973; Mawson et al., 2000). Evidently, there is a gap in the research assessing if low to moderate altitude exposure alters RMR in athletic cohorts, and if the response changes with acclimatisation. Physiological adaptations with altitude exposure may also introduce biological variability into RMR measurements that occur at sea-level post-altitude exposure. An early study reported unchanged RMR in men and women following 12 days of exposure to 1850 m (Terzioglu & Aykut, 1954). However, RMR was not measured until 4-5 days post-altitude exposure (Terzioglu & Aykut, 1954). A case study involving elite male and female rowers observed a trend for reduced RMR (~5%) on return from a 12-day training camp at altitude (~1800 m) (Woods, Garvican-Lewis, et al., 2017). As loss of FM also occurred, this reduced RMR was attributed to LEA exposure in the absence of a controlled EI during the camp (Woods, Garvican-Lewis, et al., 2017). However, failure to control for or measure EA prevents the ability to discern the effects of altitude versus LEA exposure on RMR. No other studies could be located look at the impact of recent altitude exposure on RMR.

2.2.5 Season

Seasonal variations in RMR have been reported with a greater RMR in the winter compared to summer months for those living in Japan (~16%) (Osiba, 1957), Korea (~11-24%) (Kang et al., 1963; Park et al., 1969; Pham et al., 2020), and Northeastern Siberia (~6%) (Leonard et al., 2014). Similarly, SMR is greater in the winter compared to summer for men and women living in Netherlands (Plasqui et al., 2003). However, findings of seasonal variations in RMR are not consistent, with no difference in the summer and winter BMR measurements of men living in United Kingdom (Haggarty et al., 1994). Interestingly, seasonal changes in RMR have been reported in Korean women that engage in diving year-round wearing only a bathing suit, but not in Korean women non-divers, suggesting that the increased RMR seen in the winter was an adaptation to repeated cold water immersion (Kang et al., 1963). This may have implications for athletes living in locations with seasonal variations in temperature but not exposing themselves to different temperatures across seasons. The season that RMR measurements occur in should be noted when measuring an athlete's RMR until the extent to which this contributes variability in RMR measurements is determined.

2.3 Sources of error

This final section will discuss sources of error in RMR measurements (see Figure 2.4). Failing to control for these factors may lead to falsely elevated or lowered RMR measurements that may in turn lead to a false positive or negative diagnosis of LEA.

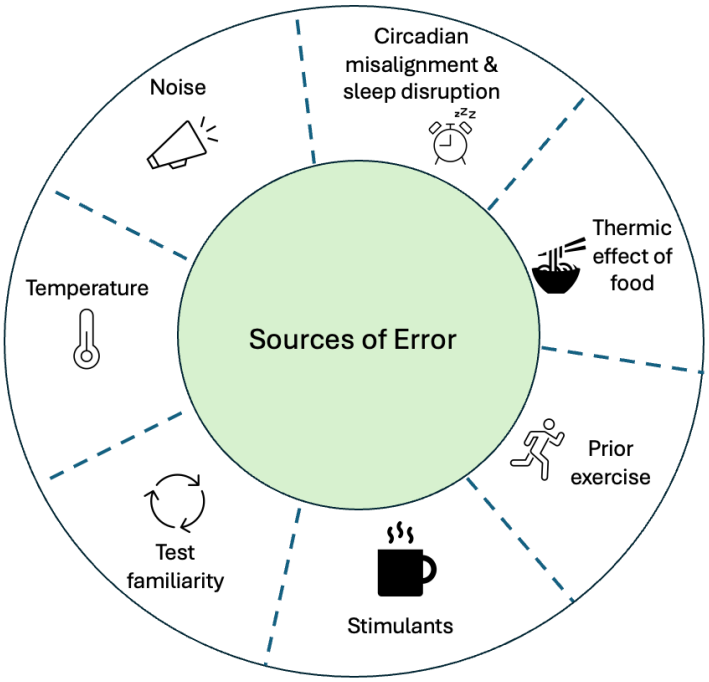

Figure 2.4. Potential sources of error in RMR measurements.

2.3.1 Thermic effect of food

The post-prandial increase in metabolic rate due to the digestion, absorption and metabolism of dietary nutrients is known as TEF (Calcagno et al., 2019). While it typically peaks between ~60-180 minutes post-prandial (Compher et al., 2006), various factors influence the magnitude of TEF, such as the macronutrient composition of the meal (Calcagno et al., 2019). To avoid an artificially elevated RMR due to residual TEF, current RMR protocols recommend a fast of >7 hours (Fullmer et al., 2015) and >5 hours (Compher et al., 2006) prior to the commencement of measurement. While these guidelines target the duration between the last meal and RMR measurement, they do not consider whether the cumulative TEF over the previous 24 hours has a carry-over effect on RMR measurements taken in an overnight fasted state. This is especially pertinent in situations of high EI typical of many elite endurance athletes undertaking large training volumes, which would be expected to increase the magnitude of the TEF (Quatela et al., 2016). For example, a study from 1980 demonstrated that 24 hours of overfeeding (+68%;

3300 kcal) in men and women resulted in a ~12% increase in BMR that was measured in a 14-15 hour fasted state (Dauncey, 1980). Additionally, increases in RMR measured >9 hour post-prandially in chronic overfeeding studies, that could not be explained by changes in body composition, have also been attributed to a persistent TEF from large evening meals (Diaz et al., 1992; Joosen et al., 2005).Yet, the energy content of these "overfeeding" diets (3300-4700 kcal·day^{-1}) (Dauncey, 1980; Diaz et al., 1992; Joosen et al., 2005) believed to create artifacts in RMR measurements from residual TEF may be similar to an athletes' energy requirements during phases of high training loads (Heydenreich et al., 2017). These studies suggests that total EI in the 24 hours prior may impact next day RMR measurements even if occurring >5-7 hours post-prandially as per current guidelines (Compher et al., 2006; Fullmer et al., 2015). As such, it is unknown if an athlete's diet needs to be standardised in the 24 hours prior to a RMR measurement to control for residual TEF.

2.3.2 Stimulants

Many of the stimulants known to artificially inflate RMR measurements are banned from competition by anti-doping regulations. As such, this review will focus on nicotine and caffeine given their use in athletes.

2.3.2.1 Nicotine

Nicotine consumption acutely increases RMR (Audrain et al., 1991; Collins et al., 1994, 1996; Klesges et al., 1991; Perkins et al., 1989, 1990; Perkins & Sexton, 1995; Walker et al., 1992; Walker & Kane, 2002; Warwich et al., 1987), even with passive exposure (Metsios et al., 2007). To avoid an artificially increased RMR due to nicotine consumption, current guidelines recommend abstaining from nicotine for >120-140 minutes prior to a RMR measurement (Compher et al., 2006; Fullmer et al., 2015). Since RMR measurements are ideally undertaken in athletic populations soon after waking in the morning, such recommendations are somewhat moot. Current evidence suggests that nicotine consumption on the evening prior to a RMR

measurement is unlikely to create artifacts in morning measurements. For instance, smoking 24 cigarettes over 24 hours with the last cigarette consumed at 20:30h had no effect on morning BMR measurements (Hofstetter et al., 1986). No change in BMR was reported following periods of smoking cessation (Hellerstein et al., 1994; Warwick et al., 1995). However, this is not a consistent finding, with one study reporting a decrease in RMR following 30 days of smoking cessation (Moffatt & Owens, 1991). Notably, only one study has investigated the effect of electronic cigarettes, an increasingly popular form of nicotine exposure in young people, on RMR; no change was detected when measured 40 minutes after the last electronic cigarette inhalation (Fogt et al., 2016). As a precaution in both research and clinical settings, the absence of nicotine consumption on the morning of a RMR measurement should be checked, especially in athletic populations with higher prevalence of nicotine use, such as ice hockey, football, rugby, and wrestling (Marclay et al., 2011).

2.3.2.2 Caffeine

Numerous studies have demonstrated an increase in RMR following caffeine consumption (Acheson et al., 1980; Arciero et al., 1995, 2000; Astrup et al., 1990; Belza et al., 2007; Collins et al., 1994; Dulloo et al., 1989; Hamada et al., 2008; Hollands et al., 1981; Jung et al., 1981; Koot & Deurenberg, 1995; LeBlanc et al., 1985; Liu et al., 2015; Pérez et al., 2021; Poehlman et al., 1985) with increases being reported with caffeine doses as small as 50 mg (Belza et al., 2007) persisting for up to 4 hours post-consumption (Belza et al., 2007; Liu et al., 2015). This effect seems to occur independently of an individual's habituation to caffeine consumption (Poehlman et al., 1985). Current guidelines recommend abstaining from caffeine consumption for at least 4 hours prior to RMR measurements (Compher et al., 2006; Fullmer et al., 2015), but like nicotine consumption, there is no clear conclusion as to how long RMR stays elevated after caffeine consumption. The half-life of caffeine is variable with a range from ~3-10 hours (Grant et al., 2018) as its metabolism is influenced by multiple factors, such as genetics (Nehlig,

2018), OCP usage or MC phase (Abernethy & Todd, 1985; Balogh et al., 1995; Lane et al., 1992; Patwardhan et al., 1980). Although it is possible that residual caffeine is present with prolonged periods of abstention (e.g. detectable caffeine levels were found in 66% of participants prior to cardiac testing even after 24 hours of abstention (Zheng & Williams, 2002)), there is no evidence that this has a meaningful impact on RMR. For example, in a cohort of women that were habitual caffeine users, 20 $mg \cdot kg^{-1} \cdot day^{-1}$ of caffeine consumed via 5 cups of coffee with the last dose at 19:15h had no impact on BMR measured the following morning (Bracco et al., 1995). Similarly, no change in RMR was seen following 4 days of 5 $mg \cdot kg^{-1} \cdot day^{-1}$ of caffeine split across a morning and afternoon dose in male non-caffeine consumers (Júdice et al., 2013). Given that RMR measurements occur in the morning after an overnight fast, it is unlikely that athletes will have consumed caffeine within the ~8-hour period prior to testing. Indeed, this would only occur if caffeine were consumed just prior to bed, in contradiction to current guidelines surrounding caffeine and sleep in athletes which recommend that caffeine consumption is stopped >9 hours prior to bed (Gardiner et al., 2023). Like nicotine, it seems unlikely that residual caffeine intake from the day prior will create artifacts in RMR measurements.

2.3.3 Prior exercise

As most measurements of metabolic rate are done using an outpatient protocol, it is recommended that a rest period is implemented before initiating a RMR measurement to allow any elevations in RMR due to commuting to the testing site to subside (Compher et al., 2006; Fullmer et al., 2015). Studies which have compared outpatient and inpatient protocols of RMR assessment have reported similar RMR measurements when a ~25-30-minute rest period is implemented prior to RMR being measured in an outpatient setting (Bone & Burke, 2018; Turley et al., 1993). Additionally, ~20 minutes appears to be sufficient to return RMR to baseline values following a 300 m walk (Frankenfield & Coleman, 2009). In addition to

implementing an acute rest period, purposeful exercise is restricted prior to the RMR measurement to avoid the elevations in metabolic rate which remain after exercise cessation, known as EPOC (Børsheim & Bahr, 2003). Although it is known that EPOC is greatest immediately following exercise, then progressively declines (Speakman & Selman, 2003), there is no clear consensus on how long exercise should be restricted prior to a RMR measurement (Fullmer et al., 2015). Indeed, the time required for metabolic rate to return to baseline will depend on the intensity and duration of the exercise task (Laforgia et al., 2006; Panissa et al., 2021). Table 2.5 highlights studies that have measured RMR before and in the days following an acute bout of exercise in an overnight fasted state with findings of an increased RMR post-exercise suggesting that EPOC resulted in an artificially inflated RMR measurement. Of the nine available studies of changes in RMR following low to moderate intensity continuous exercise, five demonstrated an elevated RMR measurement 18-22 hours post-exercise (Bielinski et al., 1985; Francois et al., 2017; Hunter et al., 2017; Jamurtas et al., 2004; Maehlum et al., 1986). Studies that failed to detect an impact on post-exercise RMR tended to be of shorter duration (<50 minutes) and all involved cycling as an exercise modality (Almuzaini et al., 1998; Gillette et al., 1994; Greer et al., 2015; Kolkhorst et al., 1994; Wilmore et al., 1998). Results from studies assessing changes with acute bouts of high intensity interval training (HIIT) and resistance exercise are more consistent, with most showing an increase with both HIIT (Francois et al., 2017; Greer et al., 2015; Hunter et al., 2017) and resistance exercise (Burt et al., 2014; Dolezal et al., 2000; Gillette et al., 1994; Greer et al., 2015; Hackney et al., 2008; Melby et al., 1993; Osterberg & Melby, 2000; Paoli et al., 2012; Paschalis et al., 2010; Schuenke et al., 2002). Only one study assessing HIIT exercise found no change in RMR (Greer et al., 2021), while only two studies failed to detect a change in RMR in response to an acute bout of resistance exercise (Abboud et al., 2013; Greer et al., 2021). Caveats to this literature, however, include the low athletic calibre of study participants, noting that training

status influences the time needed for metabolic rate to return to baseline following an acute bout of exercise (Short & Sedlock, 1997). Other concerns include the marked lack of female participants within these studies, and failure of most exercise protocols to reach the workloads typically undertaken by high calibre athletes. It should be noted that fluctuations in RMR have been reported across match week in soccer players (Carter et al., 2023) and rugby union players, with an increased RMR following game day (Hudson et al., 2020) being attributed to muscle damage with competition. Evidently, this is an area of high research priority given its higher pertinence to athletic cohorts.

Table 2.5. Impact of an acute bout of exercise on post-exercise RMR measurements.

Low to moderate continuous exercise			
Reference	Participant information	Exercise intervention	Results
Bielinski et al., 1985	n= 10M Physically active VO_2 max: 62.5±2.2 ml/kg/min	3 hrs running at 50% VO_2 max	4.7% increase 18 hrs post-exercise
Maehlum et al., 1986	n= 4M/4F VO_2 max~ 47 ml/kg min	80 min cycling at 70% VO_2 max	Increased 24 hrs post-exercise
Kolkhorst et al., 1994	n= 9M Physically active VO_2 max: 49±6 ml/kg/min	45 min running or cycling at 60% VO_2 max x 3 consecutive mornings	No change on the 7 consecutive mornings post-exercise
Wilmore et al., 1998	n= 40M/37F Measurement at end of 20 wk endurance training program	50 min cycling at 75% of VO_2 max	No change 24 hrs and 72 hrs post-exercise
Almuzaini et al., 1998	n= 10M Physically active VO_2 max: 40.0±2.2 ml/kg/min	Cycling at 70% of VO_2 max for 30 min or 2x15 min sessions	No change 18 hrs post-exercise
Jamurtas et al., 2004	n= 10M VO_2 max~ 51 ml/kg/min	60 min running at 70-75% of VO_2 max	7.1% increase 48 hrs post-exercise but not elevated at 24 hrs or 72 hrs post-exercise
Greer et al., 2015	n= 10M VO_2 max: 34.5±6.1 ml/kg/min	40 min cycling at 40% VO_2 max	No change 21 hrs post-exercise

Hunter et al., 2017	n= 33F Sedentary VO_2 max: 25.3±6.1 ml/kg/min Engaging in 16 wk exercise program	60 min cycling at 50% VO_2 max	64 kcal/day increase 22 hrs post-exercise Increase seen both before and after 16 wk training program
Francois et al., 2017	n= 12M Untrained VO_2 max: 44±8 ml/kg/min	50 min cycling at 65% VO_2 max	Increased 22 hrs post-exercise

High intensity interval training

Reference	Participant information	Exercise intervention	Results
Greer et al., 2015	n= 10M VO_2 max: 34.5±6.1 ml/kg/min	30 sec cycling bouts at 90% VO_2 max (~40 min total)	Increased 21 hrs post-exercise
Francois et al., 2017	n= 12M Untrained VO_2max: 44±8 ml/kg/min	5 x 30 sec Wingate's	Increased 22 hrs post-exercise
		Repeated sprints with arm crank	Increased 22 hrs post-exercise
Hunter et al., 2017	n= 33F Sedentary VO_2 max: 25.3±6.1 ml/kg/min Engaging in 16-wk exercise program	~145 sec cycling bouts at 85% VO_2 max	103 kcal/day increase 22 hrs post-exercise Increase seen both before and after 16 wk training program
Greer et al., 2021	n= 7F Physically active VO_2 max: 50.9±4.1 ml/kg/min	20 x 30 sec running at >90% VO_2 max	No change 24 hrs post-exercise

Resistance exercise

Reference	Participant information	Exercise intervention	Results
Melby et al., 1993	n= 7M Resistance trained	6 sets of 10 weightlifting exercises at 70% of 1RM with 8-12 reps/set	9.4% increase 14 hrs post-exercise

Gillette et al., 1994	n= 10M Physically active VO_2 max: 52.0±8.5 ml/kg/min	5 sets of 10 weightlifting exercises	Increased 14.5 hrs post-exercise
Dolezal et al., 2000	n= 9M untrained n=9M trained	8 sets of leg press at 6-RM	Increased 24 hrs and 48 hrs post-exercise, but not 72 hrs post-exercise Greater increase in untrained than trained
Osterberg et al., 2000	n= 7F Physically active	5 sets of 10 different weightlifting exercises at 70% of 1-RM with 10-15 reps/set	4.2% increase 16 hrs post-exercise
Schuenke et al., 2002	n= 7M Resistance trained	30 min circuit of resistance training involving 3 exercises with 4 sets and 8-12 reps/set	Increased 14 hrs and 38 hrs post-exercise
Jamurtas et al., 2004	n= 10M VO_2 max~ 51 ml/kg/min	Resistance exercise session at 70-75% of 1RM	5.5% increase 24 hrs post-exercise but no longer elevated at 48 hrs or 72 hrs post-exercise
Hackney et al., 2008	n= 8M untrained n= 8M resistance trained	8 types of resistance exercise with 5 sets per exercise	Increased for both untrained and trained participants at 24 hrs, 48 hrs, and 72 hrs post-exercise At 72 hrs post-exercise, RMR increased by 9.2% for untrained, and 7.9% for resistance trained
Paschalis et al., 2011	n= 20F	5 sets of 15 maximal voluntary contractions seated knee extensions with concentric concentration (n= 10) or eccentric concentration (n=10) Performed once/wk for 8 wks	No change 48 hr post-exercise with concentric concentration 12.7% increase 48 hrs post-exercise, but only prior to beginning 8 wk training with eccentric concentration
Paoli et al., 2012	n= 17M Resistance trained	High-intensity interval resistance Traditional resistance training	23.4% increase 22 hrs post-exercise Greater increase compared to traditional 5.2% increase 22 hrs post-exercise

Abboud et al., 2013	n= 8M Resistance trained	Resistance exercise with load volume of 10,000 kg or 20,000 kg	No change 24 hrs or 48 hrs post-exercise
Burt et al., 2014	n= 8M Endurance trained but not resistance trained VO_2max: 54.2±5.5 ml/kg/min	100 squats	11.8% increase at 24 hrs and 13.2% increase at 48 hrs post-exercise
Greer et al., 2015	n= 10M VO_2 max: 34.5±6.1 ml/kg/min	Resistance exercise session (~45 min)	Increased 21 hrs post-exercise
Greer et al., 2021	n= 7F Physically active VO_2 max: 50.9±4.1 ml/kg/min	30 min circuit resistance exercise	No change 24 hrs post-exercise

RMR resting metabolic rate; VO_2 max maximal aerobic capacity; RM repetition maximum; HIIT high intensity interval training.

2.3.4 Testing familiarity

Erroneous RMR measurements may occur due to unfamiliarity with the test. For example, in studies involving repeated measurements of RMR, the large variability in initial RMR measurements has been attributed to the time required to adapt to the indirect calorimetry equipment (Leff et al., 1987). A period of familiarisation with equipment prior to commencing a RMR measurement may help to overcome this. Indeed, women who performed three practice visits (resting supine with mouthpiece plus nose clip for 10 minutes) or were acclimatised with the mouthpiece plus nose clip for 5 minutes on the day of testing prior to commencing a RMR measurement had a lower absolute RMR than a control group (Practice ~7.6% decrease; Acclimatisation ~9.5% decrease compared to control) (Scott, 1993). Yet, this is not a consistent finding as the day-to-day variability did not decrease with familiarisation, when RMR was measured 5 times within a 2-week period using the hood or mouthpiece plus nose clip (Roffey et al., 2006). These conflicting results may relate to differing protocols. For the study finding no effect of familiarisation, RMR was measured for 15 minutes and 10 minutes of the lowest CV for VO_2 was used to calculated RMR (Roffey et al., 2006) whereas in the study finding an effect of familiarisation, RMR was measured for 5 minutes following the acclimatisation with all data being used to calculate RMR (Scott, 1993). While familiarity with equipment may improve measurement reliability, suggesting the benefits of longer tests which allow early data to be discarded, this must be balanced with the potential for error to be introduced due to discomfort with prolonged exposure to equipment. For instance, a study comparing gas collection devices found that the mouthpiece plus nose clip became less reliable over a 50-minute measurement whereas the other devices became more reliable (Isbell et al., 1991). Although a 15-minute familiarisation with a mouthpiece is typically implemented prior to commencing a 20-minute measurement (Bone & Burke, 2018),

it is possible that prolonged exposure to the mouthpiece plus nose clip creates discomfort-induced errors. Notably, the impact of discomfort on RMR measurements is not well studied. While an extreme example, it has been noted that RMR measurements were increased when a muscle biopsy was performed under local anaesthesia (Damask et al., 1983). Research is needed to determine if discomfort creates artifacts in RMR measurements as well as the most suitable length for a familiarisation period to overcome error from unfamiliarity with the measurement. Regardless, an athlete's first RMR measurement should be interpreted with caution as unfamiliarity with the measurement may create artefacts in results.

2.3.5 Temperature

The thermoneutral zone is the temperature range at which conductance altering mechanisms alone are able to maintain heat production to heat balance without physiological adaptations to reduce heat loss or increase heat production (Brychta & Chen, 2017). Within this zone, RMR is at its lowest (Cannon & Nedergaard, 2011), whereas at temperatures below the thermoneutral zone, there is an increase in RMR to help maintain a stable core body temperature (Brychta & Chen, 2017). This is known as cold-induced thermogenesis and includes shivering to initiate heat production and non-shivering thermogenesis (Cannon & Nedergaard, 2011). A ~7-14% increased RMR has been reported in temperatures of 15ºC-16ºC compared to 22ºC (van Marken Lichtenbelt et al., 2009; Van Ooijen et al., 2004) as well as 15ºC compared to 28ºC (Claessens-Van Ooijen et al., 2006). While 28-32ºC is typically defined as the thermoneutral zone, the lower critical temperature is highly individual and dependent on various biological factors (Brychta & Chen, 2017; Pallubinsky et al., 2019) as well as season (Kashiwazaki et al., 1990; Nishimura et al., 2015; Van Ooijen et al., 2004). Notably, these studies exposed participants to varying temperatures with minimal clothing and no blanket/duvet during the RMR measurement. This may have minimal

application to RMR measurements as current guidelines recommend providing a blanket during the measurement if room temperature is not maintained at 22-25ºC (Fullmer et al., 2015). The effect of temperature above the thermoneutral zone on RMR is not as well studied. While not a consistent finding (Pallubinsky et al., 2019), a greater RMR at 40ºC compared to 28ºC has been reported (Færevik et al., 2001; Henderson et al., 2021) with the ~35% increase in RMR at 40ºC increasing a further ~15% at 50ºC (Henderson et al., 2021). This increased RMR was seen without an increase in core temperature and is thought to be due to an increased heart rate, minute ventilation, and sweat rate (Henderson et al., 2021). However, the extreme temperature of 40ºC may also have minimal application to RMR measurements as most locations that reach this temperature would have a temperature-controlled room. RMR measurement rooms should be maintained at a temperature of 22-28ºC, but given that the thermoneutral zone is highly individual, athletes should be given a blanket to maintain a comfortable body temperature and be guided to wear clothing that is suitable for the temperature of the room.

2.3.6 Noise and distraction

RMR measurements typically occur in a room with minimal sounds or distractions with the requirement that athletes stay awake. Yet, the impact of background noise and distractions on RMR measurements is conflicting. One study reported significantly higher RMR measurements when male and female participants were reading (~6.5% increase) or listening to music (~1.8% increase) compared to a control condition (Snell et al., 2014). Contrary to these findings, another study found no difference in measured RMR when listening to classical music or self-selected music compared to a control condition (Splinter & Wilson, 2019) or when listening to stressful music or calm music compared to a control condition (Carlsson et al., 2005). Notably, if listening to music does not impact RMR, this may be a method to improve measurement reliability for

athletes that struggle to stay awake during RMR measurements or become irritable/restless during measurements due to boredom. It must also be considered that the sound of consistent music during a RMR measurement may have different outcomes than inconsistent and unexpected noises in the surrounding environment during RMR measurements, such as people talking in surrounding areas and/or doors slamming shut. As such, noise in the surrounding environment should be minimised and athletes should not be presented with distractions during a RMR measurement until it is determined if this creates artifacts in RMR measurements.

2.3.7 Circadian misalignment and sleep disruption

Circadian misalignment occurs when internal circadian timing systems are not aligned with the external environment such as fasting and feeding cycles or sleep and wake cycle and frequently occurs from shift work or jet lag (Morris et al., 2012). Most studies have examined the impact of circadian misalignment on SMR rather than RMR with findings of both an increased (Gonnissen et al., 2012; Wefers et al., 2018) as well as decreased (McHill et al., 2014) metabolic rate during daytime sleep. A study of men and women found no effect of circadian misalignment on RMR measured in the biological evening or morning (Morris et al., 2015). On the other hand, sex-based differences have been observed with circadian misalignment, with women showing a 4.5% increase in RMR whereas no change was observed in males with circadian misalignment (Qian et al., 2019). The sleep disruption and restriction that occurs with circadian misalignment must also be considered. Three weeks of sleep restriction and circadian misalignment led to an 8% decrease in the RMR of men and women (Buxton et al., 2012). However, most studies examining the impact of sleep restriction alone show no impact on RMR (Bosy-Westphal et al., 2008; Covassin et al., 2022; Grant et al., 2022; Nedeltcheva et al., 2009; Shechter et al., 2014; St-Onge et al., 2011). One study did report a 3% decrease in RMR when sleep was restricted to 4 hours per night for 5 nights

with RMR returning to baseline after 1 night of 12 hours of recovery sleep (Spaeth et al., 2015). While there is insufficient evidence to suggest that sleep disruption will create artifacts in RMR measurements, there is evidence to suggest that circadian misalignment may introduce error. As such, RMR measurements should be scheduled away from travel that induces jet lag and shiftwork.

2.4 Conclusion

Evidently, there are numerous biological, technical and environmental factors that contribute to variability in RMR measurements as well as factors that lead to erroneous RMR measurements. While the influence of some of these factors are well studied, other areas of high pertinence to the athlete, have received little attention. The outputs of this PhD program will seek to elucidate factors contributing to variability in RMR measurements as well as artifacts that may interfere with a valid and clinically useful interpretation of the data. Given that some gaps in the literature are beyond the scope of one PhD program, factors that are of high priority to the athlete will be addressed. The experimental chapters in this thesis will provide insight into 1) The impact of MC phase and HC usage on RMR; 2) The effect of altitude exposure on RMR, and if LEA alters this response; 3) The need to control exercise and diet the day prior to a RMR measurement; 4) Barriers and enablers to measuring RMR in a high-performance sport environment. While it is acknowledged that RMR measurements are also a useful tool to monitor the health status of male athletes, this PhD program also seeks to address gender gaps within exercise science research. To aid in minimising this research gap, all research projects will include female only cohorts or for mixed study cohorts involve sex-based comparisons.

3 Methodology and Design

In keeping with Australian Catholic University guidelines, the methods utilised within each study of this thesis are described in full in the current chapter. However, in chapters 4-8, the methods section for each study is written as per the guidelines of the respective journal. Here, studies 1-3 will be discussed together given their shared methodological overlap. The methodology of study 4 (see chapter 8) will be discussed separately given its qualitative research design.

3.1 Participants and study design

3.1.1 Study 1

Participants for this study included 25 female athletes from the National Rugby League's Indigenous Women's Academy of Tier 3 calibre (Highly trained/National level) (McKay et al., 2022), and included a mixture of NC athletes (n=11) and HC users (n=14). These athletes were recruited for a 5-week training camp at the Australian Institute of Sport (AIS). 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 standardised protocol for body composition and RMR measurements. 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 (DMPA).

NC athletes tracked their MC for 11 weeks prior to study commencement, using an online reporting system (REDCap). This also involved confirming the surge in LH that occurs prior to ovulation via urinary LH surge testing with urinary ovulation kits (Advanced Digital Ovulation Test, Clearblue, Geneva, Switzerland). NC athletes tested for the surge in LH from day 8 of the MC

until the surge in LH had been confirmed or until day 17, if the LH surge 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: 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. As such, each athlete underwent measurements at 3 time points during the 5-week training camp (see Figure 3.1). Alongside RMR measurements, athletes underwent a dual energy x-ray absorptiometry (DXA) scan to estimate body composition and following these measurements a blood draw was taken.

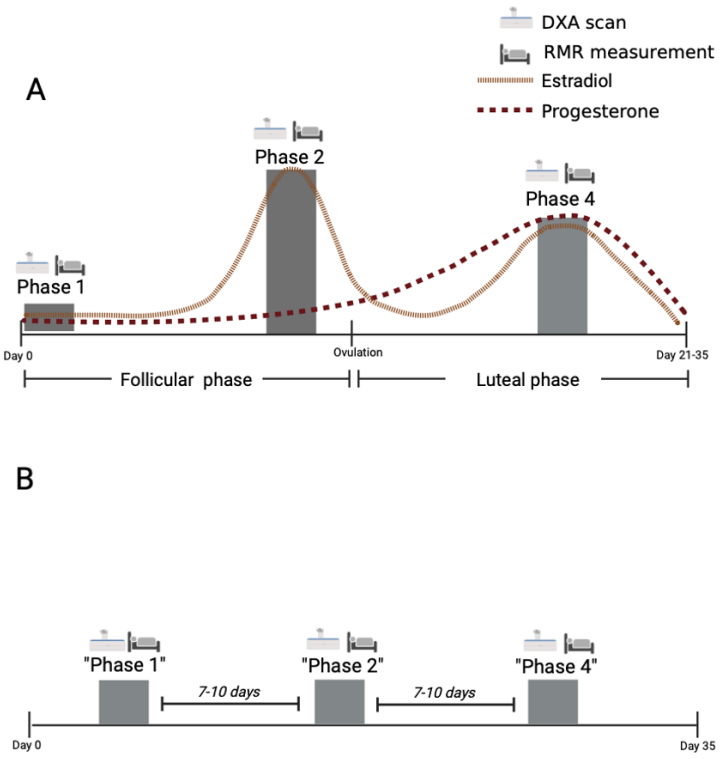

Figure 3.1. Overview of experimental protocol for study 1. Measurements occurred during Phase 1, Phase 2, and Phase 4 of the menstrual cycle in naturally cycling athletes (A) and during three spaced occasions for hormonal contraceptive users. For combined-monophasic oral contraceptive users, testing occurred on active pill-taking days. For all other hormonal contraceptive users (injection and implant), testing occurred at any given time (B). DXA, dual energy X-ray absorptiometry; RMR, resting metabolic rate.

3.1.2 Study 2

Participants for this study included twenty female race-walkers (26.5±6.5 years, VO_2max: 58.2±4.2 ml·kg^{-1}·min^{-1}) of Tier 3 (Highly Trained/National Level) to Tier 5 (World Class) calibre (McKay et al., 2022). Athletes included both NC athletes (n=13) and HC users (n=6 OCP, n=1 Implanon). It was not possible to standardise MC or HC phase within RMR measurements because the research-embedded training camp study design required that all athletes needed to travel to

altitude and begin the study at the same time. However, the menstrual status of each athlete was still characterised with consideration of the Best Practice Guidelines (Elliott-Sale et al., 2021). Data of one NM athlete were excluded from analysis due to an injury sustained during the first week at altitude, thus preventing full completion of the study.

Baseline testing occurred at the AIS (~580 m) over a 5-day period during which time all athletes had standardised dietary control. Athletes then travelled by vehicle to Perisher Valley, Australia (~1800 m) for a 3-week altitude training camp before returning to the AIS for post-altitude testing that occurred over a 4-day period (See Figure 3.2). The first 2 weeks at altitude served as an acclimatisation period during which time all athletes consumed a fully provided diet providing an EA of 45 kcal·kg FFM^{-1}·day^{-1}. This was followed by a 7-day dietary intervention, which manipulated EA. During this dietary intervention, one group of athletes (n=10) consumed a diet providing an EA of 15 kcal·kg FFM^{-1}·day^{-1} (LEA) while the remaining athletes (n=9) continued to consume a diet providing an EA 45 kcal·kg FFM^{-1}·day^{-1} (high energy availability; HEA). Athletes were allocated into groups based on individual preferences for the EA intervention, with athletes who nominated no preference allocated strategically to ensure key characteristics (e.g., menstrual status, athlete calibre, etc.) were balanced between dietary groups. In order to assess the time course of potential changes in RMR at altitude, athletes in the HEA group had RMR measured pre-altitude exposure during the baseline testing period (Pre-alt), after ~36 hours exposure to altitude (36h-alt), 2 weeks altitude exposure (Wk2-alt), 3 weeks altitude exposure (Wk3-alt), and ~36 hours post-altitude (36h-post). To assess the impact of LEA on RMR measurements, athletes in the LEA group had RMR measured at Pre-alt, and before and after the dietary intervention, which corresponded to a RMR measurement at Wk2-alt and Wk3-alt. In recognition of the burden

already associated with the LEA diet, athletes in the LEA group were not required to undergo additional RMR measurements at 36h-alt, and 36h-post. Body composition was also assessed using DXA at Pre-alt and 36h-post. A blood draw was taken at Pre-alt, Wk3-alt and 36h-post in both group of athletes to measure sex hormones and indicators of LEA.

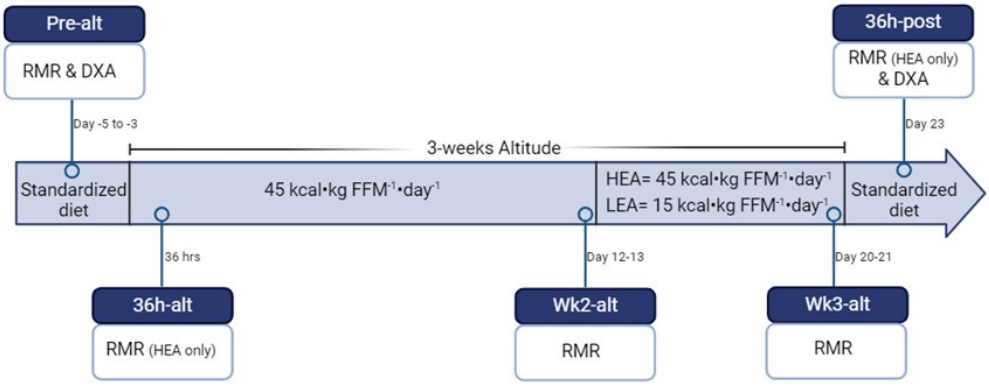

Figure 3.2. Overview of experimental protocol for study 2, detailing elevation, timeline, dietary protocols, and measurements taken. FFM, fat free mass; RMR, resting metabolic rate; DXA, dual-energy-x ray absorptiometry; HEA, high energy availability; LEA, low energy availability.

3.1.3 Study 3

Participants for this study included ten male and ten female endurance-trained athletes (n=11 cyclists, n=5 triathletes, n=3 runners, n=1 cyclist & runner) of Tier 2 (n=18) to Tier 3 (n=2) calibre (McKay et al., 2022). All female athletes were COC users with testing occurring during the active pill phase of the COC cycle. All had used COC for >3 months prior to commencing the study with the mean length of usage being 7.1±8.5 years. COC brands included: Femme-Tab (n=2), Levlen ED (n=2), Micronelle 30 ED (n=1), Evelyn 150/30 ED (n=1), Yasmin (n=1), Isabelle (n=1), Estelle-35 ED (n=1), and Yang (n=1). Seven participants habitually consumed their COC in the morning, and the remaining three in the evening. Participants that habitually consumed their COC

in the morning were required to consume their COC after completing measurements on the day of testing.

Prior to commencing the first experimental condition, athletes attended the laboratory for baseline testing. Baseline testing included a RMR measurement, DXA scan, and a maximal exercise test on a cycle ergometer. The information from these measurements was used to determine dietary and exercise targets for the 5 experimental conditions. The 5 experimental conditions included: EA that would result in BM gain if consumed chronically without exercise (GEA_{rest}; 75 kcal·kg FFM^{-1}), high EA with exercise (HEA_{ex}; 45 kcal·kg FFM^{-1}), high EA without exercise (HEA_{rest}; 45 kcal·kg FFM^{-1}), LEA with exercise (LEA_{ex}; 15 kcal·kg FFM^{-1}), and LEA without exercise (LEA_{rest}; 15 kcal·kg FFM^{-1}). Athletes underwent these 5 conditions using a Latin square counterbalance design with an average washout of 8 days between conditions. Each condition involved 3 days (see Figure 3.3). Day 1 of each condition involved 24 hours of diet and exercise standardisation during which time athletes received a diet providing an EA of 45 kcal·kg FFM^{-1}. Day 2 involved 24 hours of EI and EEE manipulation to achieve an EA target through diet alone or in combination with exercise as per the experimental condition. Conditions with the same EA but differing EEE (HEA_{ex} vs. HEA_{rest} and LEA_{ex} vs. LEA_{rest}) allowed for the effects of EPOC to be assessed; the conditions without exercise but varying EI (GEA_{rest}, HEA_{rest}, and LEA_{rest}) allowed the effect of the TEF to be assessed; and the two conditions of high EA with and without exercise (HEA_{ex} and HEA_{rest}) allowed the effect of energy flux to be assessed. On Day 3, following 24 hours of diet and exercise manipulation, athletes underwent a RMR measurement, DXA scan to estimate body composition and blood draw to measure indicators of LEA

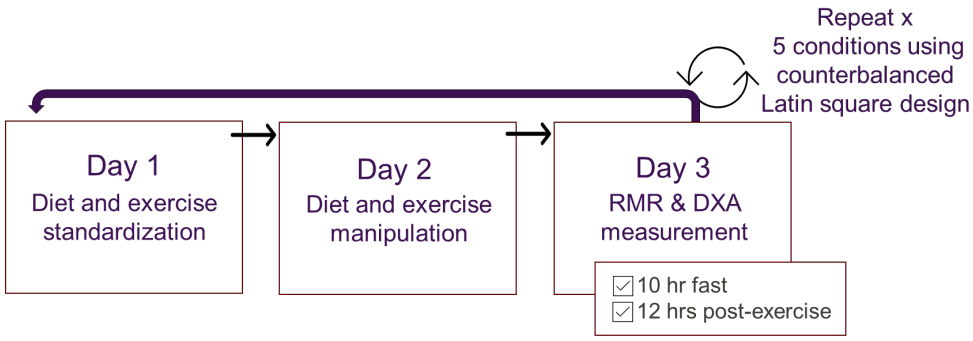

Figure 3.3. Overview of experimental protocol for study 3. Using a Latin square counterbalance design, athletes underwent five conditions of diet and exercise manipulation with each condition involving 3 days. Energy availability targets were achieved through diet alone or in combination with exercise. RMR, resting metabolic rate; DXA, dual-energy X-ray absorptiometry.

3.2 Dietary and exercise control

3.2.1 Study 1

As confirmation of MC phase was dependent on menstrual reporting that occurred mid-morning, a standardised 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 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 EI (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 standardised 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 standardised diets, and this was accounted for when analysing standardised diets.

3.2.2 Study 2

For 4 days before and 3 days after the altitude training camp, all participants consumed a standardised diet that provided ~8 g·kg $^{-1}$ carbohydrate, ~1.5 g·kg^{-1} protein and ~1.1 g·kg^{-1} fat, resulting in a daily EI of ~48 kcal·kg^{-1}. During the altitude training camp, daily energy requirements were determined prospectively for each athlete based on individualised training plans and calculated using the following equation: EI = (Target EA × FFM) + EEE. Daily protein intake was the same for both dietary interventions and provided ~2 g·kg^{-1}. When receiving a diet that contained an EA of ~45 kcal·kg FFM^{-1}·day^{-1}, ~20% of EI was from fat whereas the LEA diet provided ~15% of EI from fat. Regardless of the target EA, the remaining energy came from carbohydrates. Individual meal plans were created for each athlete based on planned training for that day and personal preference, with a chef preparing all meals.

Training load (volume x intensity) was not controlled throughout the altitude training camp. Rather, athletes followed their individualised training plans throughout the duration of the study. Daily EEE was prospectively estimated from an athlete's planned training, which included race walking, running, cycling, and/or resistance training across 1-3 sessions/day. The EEE of a race-walking training session was determined from the individualised gas exchange data collected during a 4-stage submaximal race-walking graded exercise test (GXT) completed on a treadmill during the Pre-alt period at the AIS. EEE during each GXT stage was determined using the Weir equation with Pre-alt RMR excluded from the same period as follows: [(3.94 × VO_2 + 1.11 ×

VCO_2) – (24 hr RMR/1440 (min))] (Weir, 1949). EEE per km of outdoor race walk training was then estimated from each speed of the GXT as follows: (($EEE_{kcal/min}$ × 60 min))/$Speed_{km/hr}$). Walking EEE ranged from 0.88-1.07 kcal·km^{-1}·kg^{-1} (average ~1 kcal·km^{-1}·kg^{-1}). Running EEE was estimated as kilometre ran multiplied by an athlete's BM (1 kcal·km^{-1}·kg^{-1}) (Margaria et al., 1963), cycling using a Metabolic Equivalent (MET) of 8, and resistance training a MET of 4 (Jetté et al., 1990). Pre-alt RMR was again excluded from the same time-period when estimating EEE for running, cycling and/or resistance training sessions.

Athletes reported their actual training daily to a member of the research team and EI was adjusted if the difference in EEE between actual training and planned training exceeded the EEE of 2 km of race walking. When increases in EI were needed, this was accomplished by increasing portion sizes at meals and/or providing additional snacks. When decreases in EI were needed, this was accomplished by decreasing the portion size of the day's final meal and/or removing snacks. Two days of ad libitum food intake were scheduled within the training camp: the day of ascent to altitude (day 1) and the day prior to commencing the 7-day dietary intervention after undergoing the Wk2-alt RMR measurement (day 13). These were implemented for logistical reasons and to provide participants a break from dietary control given the extensive nature and dietary compliance that this study involved.

3.2.3 Study 3

All foods and beverages were weighed and provided to athletes for both day 1 (standardisation) and day 2 (manipulation) of each condition. Athletes self-reported any deviations from the study diet. Day 1 of dietary standardisation provided an EA of 45 kcal·kg FFM^{-1} with 55% energy from carbohydrates, 25% from protein and 20% from fat. Athletes self-selected if they engaged in

exercise during this standardised period and reported this to the research team prior to the study so that EI could be set to maintain an EA of 45 kcal·kg FFM^{-1}. We allowed athletes in this study to self-select exercise on day 1 to minimise interference with an athlete's training given the extensive control of exercise that occurred on day 2 across 5 conditions. However, athletes replicated the exercise performed during the standardisation period for each of the 5 conditions. As such, an athlete's day 1 EI and EEE were the same for each of the 5 standardised periods. For day 2, EI was calculated as followed using FFM from baseline scan:

$$GEA_{rest} = 75 \text{ kcal·kg } FFM^{-1} \times FFM$$

$$HEA_{ex} = (45 \text{ kcal·kg } FFM^{-1} \times FFM) + 30 \text{ kcal·kg } FFM^{-1}$$

$$HEA_{rest} = 45 \text{ kcal·kg } FFM^{-1} \times FFM$$

$$LEA_{ex} = (15 \text{ kcal·kg } FFM^{-1} \times FFM) + 30 \text{ kcal·kg } FFM^{-1}$$

$$LEA_{rest} = 15 \text{ kcal·kg } FFM^{-1} \times FFM$$

This resulted in 3 different study diets as diets were the same for the GEA_{rest} and HEA_{ex} condition (EI= 75 kcal·kg FFM^{-1} × FFM) and the HEA_{rest} and LEA_{ex} condition (EI=45 kcal·kg FFM^{-1} × FFM) but differed in levels of EEE. The macronutrient distribution was the same for all conditions: 55% carbohydrate, 25% protein, and 20% fat. The TEF was calculated for each condition with the metabolic cost of fat being 2.5%, carbohydrate 7% and protein 27.5% (Jéquier, 2002). Athletes were required to space out meals and snacks by >1 hour, and to consume the last snack 10 hours prior to the RMR measurement so that RMR measurements always occurred in 10 hours fasted state. Athletes who were habitual caffeine drinkers were permitted to consume caffeine but were required to replicate caffeine intake across all conditions. The requirement that athletes be 10 hours fasted on the morning of day 3 further ensured that guidelines for caffeine intake prior to RMR

testing were followed given the recommendation to refrain from caffeine for >4 hours prior to RMR measurements (Compher et al., 2006).

For the two conditions involving exercise (HEA_{ex} and LEA_{ex}), the EEE of 30 kcal·kg FFM^{-1} was achieved by two bouts of cycling on a stationary load bike (Load Excalibur Sport, Groningen, Netherlands) during day 2. This included a morning bout at a wattage corresponding to 55% of VO_2max as determined during baseline testing (means: males 157±40 W, females 103±16 W) that lasted on average 135±26 minutes for male athletes and 163±37 minutes for female athletes, and an afternoon bout of cycling at 65% of VO_2max (means: males 195±46 W, females 131±19 W) for 60 minutes. The afternoon session was scheduled to end 12 hours prior to the RMR measurement. The duration of the morning session was determined as followed:

Duration= (30 kcal·kg FFM^{-1} – EEE_{kcal} of afternoon session)/$EEE_{kcal/min}$ at 55% of VO_2 max

The EEE at each intensity of cycling was prospectively determined from the gas exchange data collected during the maximal exercise test performed at the baseline visit (Weir, 1949) as such:

$$EEE\ (kcal/min) = [(3.94 \times VO_2) + (VCO_2 \times 1.11)] – (24\ h\ RMR/1440)$$

For the remaining three conditions (GEA_{rest}, HEA_{rest}, LEA_{rest}), athletes remained physically inactive for the 24 hours prior to RMR measurements. This involved no planned exercise beyond activities of daily living.

3.3 RMR measurements

3.3.1 Studies 1 & 3

RMR was measured using the Douglas bag method with athletes being in an overnight fasted and rested state. Upon arrival, athletes first rested on a bed in a dark and quiet room for 10 minutes

before being given a one-way mouthpiece 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 they were at a comfortable temperature. Following the rest and familiarisation period, expired air was collected with a mouthpiece into a gas-impermeable Douglas bag for 10 minutes for two consecutive data collection periods. Ametek Oxygen (O_2) and carbon dioxide (CO_2) analysers (VacuMed, Ventura, CA) were calibrated with two known gas concentrations 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.

3.3.2 Study 2

RMR was measured using the ParvoMedics TrueOne 2400 metabolic cart (ParvoMedics, Salt Lakes City UT, USA) with measurements occurring in an overnight fasted and rested state. Two metabolic carts were available for testing with athletes having repeat RMR measurements on the same metabolic cart. Each ParvoMedics system was calibrated with gas concentrations (15.99% O_2, 4.00% CO_2) and ventilation using a 3L syringe prior to testing. Testing occurred across two mornings with athletes presenting in an overnight fasted state and before morning training around the same time of day (± 30 minutes) to account for circadian changes in RMR (Zitting et al., 2018). Upon arrival, athletes laid in a supine position in a dark, quiet room for 10 minutes to ensure a state of rest and were then given a one-way mouthpiece that was connected to the ParvoMedics cart for a 10-minute familiarisation period. Expired air was then collected for a single 25-minute

period. Upon completion, data were exported into a Microsoft excel file. The first 2 minutes and last 2 minutes of each 25-minute period were discarded and a mean was calculated from the remaining minutes to estimate a 24-hour absolute RMR ($kcal \cdot day^{-1}$) using the Weir equation (Weir, 1949).

3.4 Body composition analysis

DXA scans were scheduled to occur immediately before or after the RMR measurement so that athletes were in an overnight fasted and rested state. DXA was used to assess body composition, using the Best Practice Protocols of the AIS involving standardised positioning (Slater, Townsend, et al., 2023). Athlete scans were performed in the same mode (GE Lunar iDXA) and analysed using GE encore by the same trained researcher to assess FFM, LBM and FM.

3.5 Indicators of low energy availability

The RMR measurement at each testing visit were used to calculate an athlete's RMR ratio using three different predictive equations and relative RMR (see table 3.1). A RMR measurement was considered suppressed if the value fell below a standardised threshold (Stellingwerff et al., 2023; Strock et al., 2020):

Table 3.1. RMR ratio and relative RMR thresholds used to indicate a suppressed RMR in studies 1-3.

RMR equation	Threshold	
HB (males)	$RMR_M \div ((66.47 + (13.75xBM) + (5.00xHt)) – (6.76xAge))$	<0.90
HB (females)	$RMR_M \div ((655.1 + (9.563xBM) + (1.850xHt)) – (4.676xAge))$	<0.90
$Cunningham_{1980}$	$RMR_M \div (500 + (22xLBM))$	<0.90
$Cunningham_{1991}$	$RMR_M \div (370 + (21.6xFFM))$	<0.92
Relative RMR	$RMR_M \div FFM$	$<30 kcal \cdot kgFFM^{-1} \cdot day^{-1}$

BM, body mass; FFM, fat free mass; HB, Harris-benedict; Ht, height; LBM, lean body mass; RMR_M, measured resting metabolic rate

For athletes in study 1, an ABM was used in the HB equation for athletes with a BM >110% of ideal BM with ABM calculated as previously described. For study 3, RMR was also predicted using DXA-estimated brain, skeletal muscle, adipose tissue, bone and residual mass (Hayes et al., 2002) with a threshold <0.94 used to indicate LEA (Strock et al., 2020). Alongside assessing for a suppressed RMR, primary, secondary and potential indicators of LEA were measured (see table 3.2). Insulin was also measured for study 2 and 3, which is a potential indicator of LEA. However, as this does not have a lower reference range, this was not used as an indicator of LEA, but rather changes over time were analysed for both studies.

Table 3.2. Primary, secondary and potential indicators of LEA measured in studies 1-3.

Severe primary	Total testosterone <8.3 $nmol \cdot L^{-1}$ (males only)
	Free testosterone <255 $pmol \cdot L^{-1}$ (males only)
	T3 <3.5 $pmol \cdot L^{-1}$
Primary	Total testosterone: 8.3-13.5 $nmol \cdot L^{-1}$ (males only)
	Free testosterone: 255-373 $pmol \cdot L^{-1}$ (males only)
	T3 3.5-4.1 $pmol \cdot L^{-1}$
Secondary	TC >5.2 $mmol \cdot L^{-1}$
	LDL >3.4 $mmol \cdot L^{-1}$
Potential	Cortisol >620 $nmol \cdot L^{-1}$
	IGF-1 below lowest quartile of age dependent range

IGF-1, insulin-like growth factor 1; LDL, low density lipoprotein; TC, total cholesterol; T3, triiodothyronine.

3.6 Blood sample analysis

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). Intra-assay CV were 5% for estradiol and 11% for progesterone. Lipids, cortisol, insulin-like growth factor 1 (IGF-1), insulin, and triiodothyronine (T3) were measured by chemiluminescent immunoassay through a commercial laboratory (Laverty Pathology, Bruce, ACT, Australia).

3.7 Statistical analysis

For all studies, statistical analyses were performed using R Studio (v3.5.2) with statistical significance accepted at an α level of $p \leq 0.05$. Statistical analyses were completed using general linear mixed models where significance of fixed effects was tested using type II Wald F tests with Kenward-Roger degrees of freedom.

3.7.1 Study 1

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. 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.

3.7.2 Study 2

For statistical analyses of RMR measurements, two separate models were used. One model assessed time course change in the HEA group only, which included test time point (Pre-alt, 36h-alt, Wk2-alt, Wk3-alt, 36h-post) as a fixed effect and subject as a random effect. The other model assessed the effect of EA manipulation, which included test time point (Pre-alt, Wk2-alt, Wk3-alt) and dietary intervention (HEA or LEA group) as a fixed effect. With this model, subject and BM were used as a random effect except for the model assessing relative RMR which only had subject as a random effect. For the models assessing diet, training, body composition, and LEA indicators, test time point and dietary intervention were fixed effects and subject was a random effect. For models assessing cortisol and T3, BM was also included as a random effect. Insulin results of two athletes (n=1 LEA athlete, n=1 HEA athlete) were considered outliers due to values being >3 SD above the mean and excluded from analyses. Non-normally distributed data (FM) was log-transformed for statistical analyses.

3.7.3 Study 3

Fixed effects for the model included condition and sex, with subject ID as a random effect within the models. BMI and subject ID were included as random effects for the models assessing relative RMR and RMR ratio with each predictive equation. Due to technical issues with the DXA machine, one female athlete could not undergo a DXA scan for the HEA_{ex} condition. The average of her DXA estimates from the remaining conditions was used for data analysis of relative RMR and RMR ratios in the general linear mixed model. However, her data was excluded from body composition analyses and when analysing the RMR ratio involving DXA estimates of tissue-organ

components. Due to technical issues obtaining the blood sample from one female for the LEA_{ex} condition, her data was excluded for blood marker analysis. Finally, one female athlete was missing T3 levels from the LEA_{rest} condition and a male athlete IGF-1 levels from the HEA_{ex} condition. For these participants, the average of remaining conditions was used for data analysis in the general linear mixed model. Non-normally distributed data (relative RMR, BM, FFM, FM, free testosterone, T3, IGF-1, insulin, and LDL) were log-transformed for statistical analyses.

3.8 Study 4

3.8.1 Participants and study design

A qualitative research design was used to investigate the use of RMR measurements in a real-world high-performance sport environment and identify potential barriers and enablers to use to inform future guideline development. A qualitative approach was taken to explore technicians' perceptions and thoughts relating to RMR measurements. A relativist ontology and constructivist epistemology methodological approach were adopted, which takes the perspective that multiple realities can exist (Willis, 2007).

Participants of this study included those employed by a National Institute Network (NIN), National Sport Organisation (NSO) or professional sporting code in Australia and measuring the RMR of athletes either in the past or presently as part of this role. Individuals at NINs, NSOs or professional sporting codes throughout Australia were contacted via e-mail, phone-call or in-person discussions from a member of the research team with details regarding the study. Recruitment was facilitated by established connections between members of the research team, and the NINs, NSOs and professional sporting codes. Initially, individuals at seven of the NINs were invited to participate, as well as eleven individuals from NSOs and professional sporting codes throughout Australia. In

situations where the individual contacted did not conduct RMR measurements, there was a request made for the contact details of the technician conducting the RMR measurement within that NSO or professional sporting code. Finally, at the end of each interview, technicians were asked if they were aware of anyone else measuring RMR in athletic cohorts in Australia. Attempts were made to interview at least one technician at each NIN, NSO and professional sporting code in Australia that measures RMR to ensure comprehensive viewpoints and experiences. All technicians provided written informed consent to participate and have interviews recorded and automatically transcribed.

3.8.2 Data collection

For technicians that consented to proceed with the study, an in-depth interview was scheduled with one member of the research team (MK) via a video call. These interviews were recorded and transcribed verbatim. The semi-structured interview schedule was developed by the research team (experts in the field), acknowledging the need for adapting questions as interviews proceeded. The interview questions were pilot tested, and feedback was used to refine the questions resulting in twenty-six questions (see appendix A of Australian Institute of Sport Insight Report on page 301 for list of questions). The average interview duration was 48 minutes.

3.8.3 Data analysis

Reflexive thematic analysis was completed to enable full data exploration and familiarisation. An inductive approach was taken, allowing researchers to identify themes from the data. The first author familiarised herself with the data by listening to interview audio-recordings following each transcript to ensure accuracy of automatic transcription and reading each transcript at least twice. Written transcripts were uploaded into NVivo 12 (QSR International, Melbourne, Australia) and initial codes generated inductively by the authors (MK and HB) and assigned to relevant text

segments. Codes were then collated into major themes and sub themes by the authors, and these themes were then reviewed and discussed with another member of the research team (HB). Any discrepancies in coding were resolved through consensus discussion among the research team. In order to provide strategies to address identified barriers and provide practical consideration for measuring RMR in the high-performance sport system, themes were mapped to the Capability, Opportunity, Motivation – Behaviour (COM-B) model and theoretical constructs of Theoretical Domains Framework (TDF) (Michie et al., 2011). The COM-B model is a framework for understanding behaviour (Michie et al., 2011). Capability refers to the technician's psychological and physical capacity to measure RMR, including having the necessary knowledge and skills, motivation refers to the brain processes that energise and direct behaviour, and opportunity is defined as factors that lie outside of the technician that make it possible to measure RMR (Michie et al., 2011). To provide further information on behaviour, the COM-B model can be mapped to the TDF, which includes 14 domains: knowledge; skills; social/professional role and identity; beliefs about capabilities; optimism; beliefs about consequences; reinforcement; intentions; goals; memory, attention and decision processes; environmental context and resources; social influences; emotions; and behavioural regulation (Cane et al., 2012).

3.8.4 Trustworthiness

Several methods were employed to enhance trustworthiness. Peer debriefing and investigator triangulation (MK and HB) were used to confirm findings and allow for different perspectives (Creswell & Miller, 2000; Spall & Austin, 1998).

4 Study 1: Effect of Menstrual Cycle Phase and Hormonal Contraceptives on Resting Metabolic Rate and Body Composition

Publication statement:

This chapter is comprised of the following paper published in *International Journal of Sport Nutrition and Exercise Metabolism*.

Kuikman, M.A., McKay, A.K.A., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Smith, E.S., McCormick, R., Tee, N., Minahan, C., Skinner, J., Ackerman, K.E. and Burke, L.M. (2024). Effect of menstrual cycle phase and hormonal contraceptives on resting metabolic rate and body composition. *International Journal of Sport Nutrition and Exercise Metabolism, 34*(4),207-217. doi: 10.1123/ijsnem.2023-0193.

The chapter does not differ from the published paper apart from tables, figures and references, which have been renumbered to maintain consistency within the thesis.

4.1 Abstract

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 analysed 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 ($p=0.028$). 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.

4.2 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 recognised as a primary or secondary indicator of Relative Energy Deficiency in Sport (REDs) in the International Olympic Committee (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.

4.3 Methods

4.3.1 Participants

The participants of this study are part of a larger study known as the Female Athlete Research Camp (FARC) with 25 female athletes 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 standardised protocol for body composition and RMR measurements. Information on the remaining NC athletes (n=11) and HC users (n=12) is summarised in Table 4.1. 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 4.1. 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.

4.3.2 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 4.1): 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).

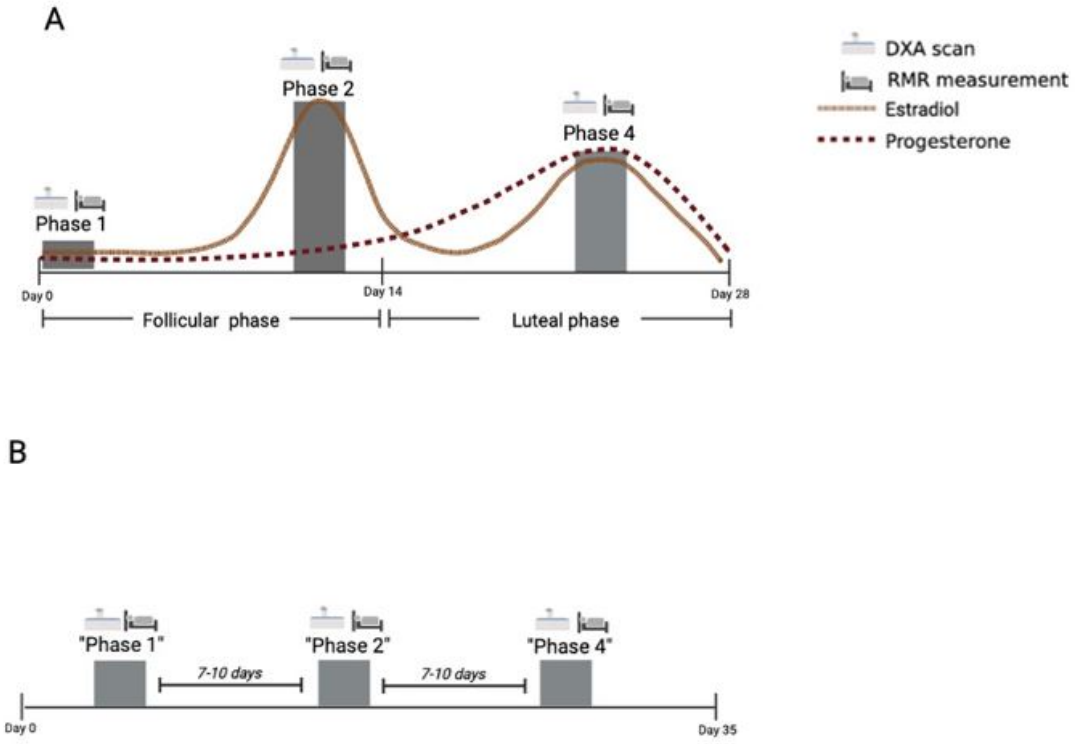

Figure 4.1. Overview of experimental protocol with measurements occurring during Phase 1, Phase 2, and Phase 4 of the menstrual cycle in naturally cycling athletes (A) and measurements occurring during three spaced occasions for hormonal contraceptive users. For combined-monophasic oral contraceptive users, testing occurred on active pill-taking days. For all other hormonal contraceptive users (injection and implant), testing occurred at any given time (B).

4.3.3 Dietary control

As confirmation of MC phase was dependent on menstrual reporting that occurred mid-morning, a standardised 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 standardised 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 standardised diets, and this was accounted for when analysing standardised diets.

4.3.4 Measurements

4.3.4.1 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 standardised positioning (Slater et al., 2023). Athlete scans were performed in the same mode (GE Lunar iDXA) and analysed 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.

4.3.4.2 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 coefficient of variation 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) analysers (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).

4.3.4.3 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 4.2 (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 4.2) (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 4.2. 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	$RMR_M \div FFM$	$<30 kcal \cdot kgFFM^{-1} \cdot day^{-1}$	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.

4.3.4.5 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).

4.3.5 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.

4.4 Results

Assessment of actual intake of the ~18-hour standardised diet showed no difference between trials or groups for energy and macronutrient intake ($p>0.05$) with actual intake achieving the standardised diet targets (Table 4.3). 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 4.3. 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.

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 analysed 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 4.4).

Table 4.4. 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

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 4.2).

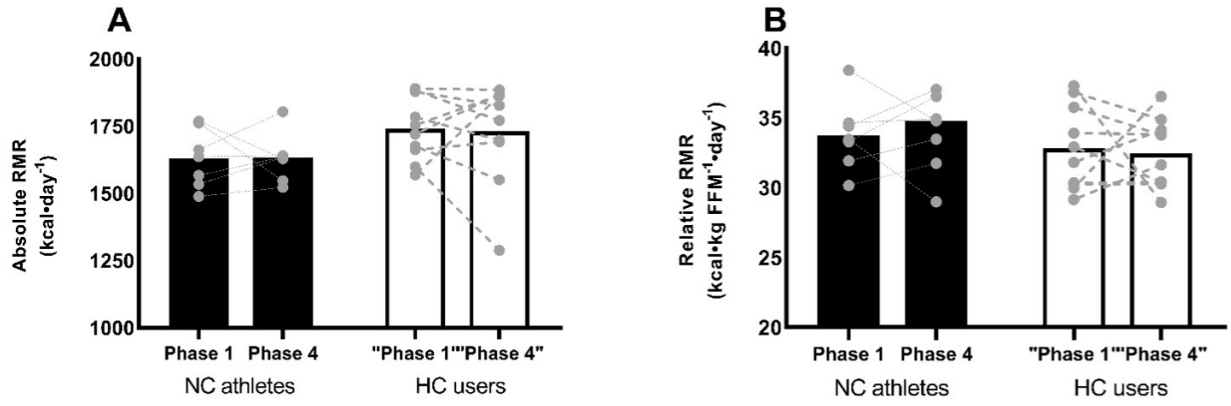

Figure 4.2. Absolute resting metabolic rate (A), and relative resting metabolic rate (B) with menstrual cycle phase and hormonal contraceptive usage. Data shown as mean with individual data points.

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 4.3).

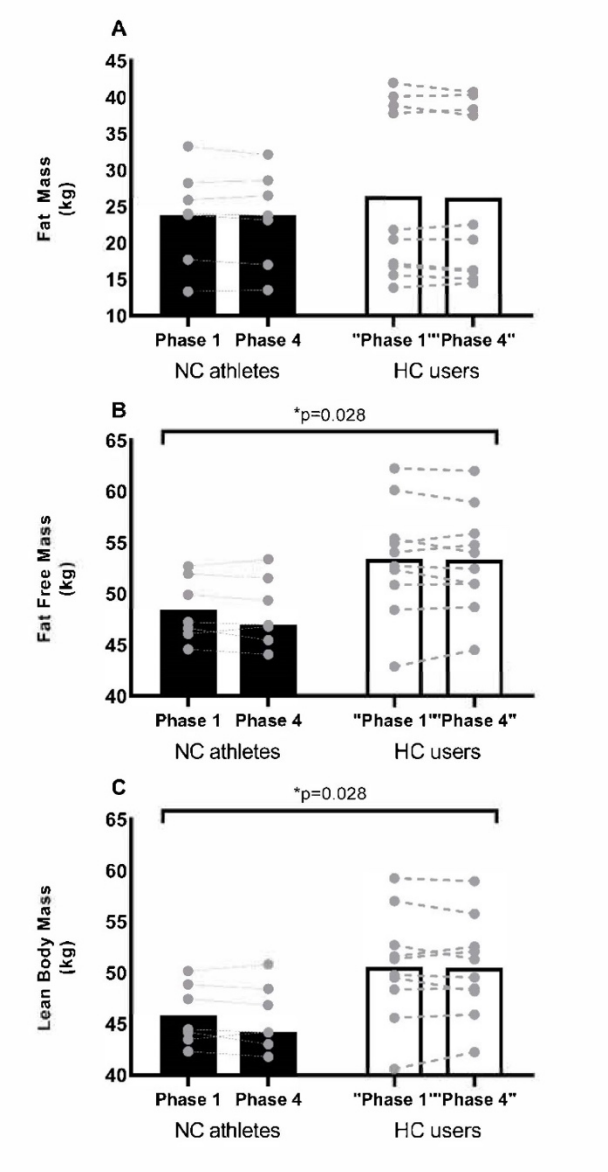

Figure 4.3. Fat mass estimates (A) fat free mass estimates (B), and lean body mass estimates (C) with menstrual cycle phase and hormonal contraceptive usage. Data shown as mean with individual data points. *Indicates significant difference between groups.

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 4.4).

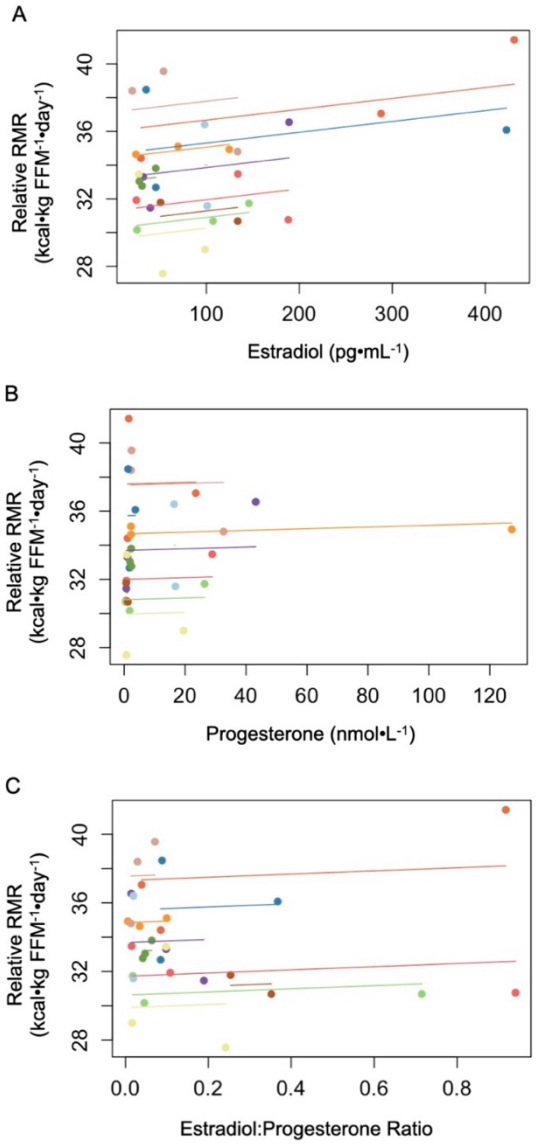

Figure 4.4. Repeated measures correlation between relative resting metabolic rate (RMR) and serum estradiol concentrations (A), serum progesterone concentrations (B) and the concentration of estradiol to progesterone ratio (C). Separate lines fit to the data from Phase 1, Phase 2, and Phase 4 measurements for each naturally cycling athlete (n=11).

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 4.5). However, the RMR ratio calculated from the HB equation using ABM was greater in HC athletes than NC athletes (p=0.020).

Table 4.5. 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.

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

4.5). No athlete presented with a RMR ratio considered suppressed when using the Cunningham$_{1980}$ or Cunningham$_{1991}$ equation or when using ABM in the HB equation.

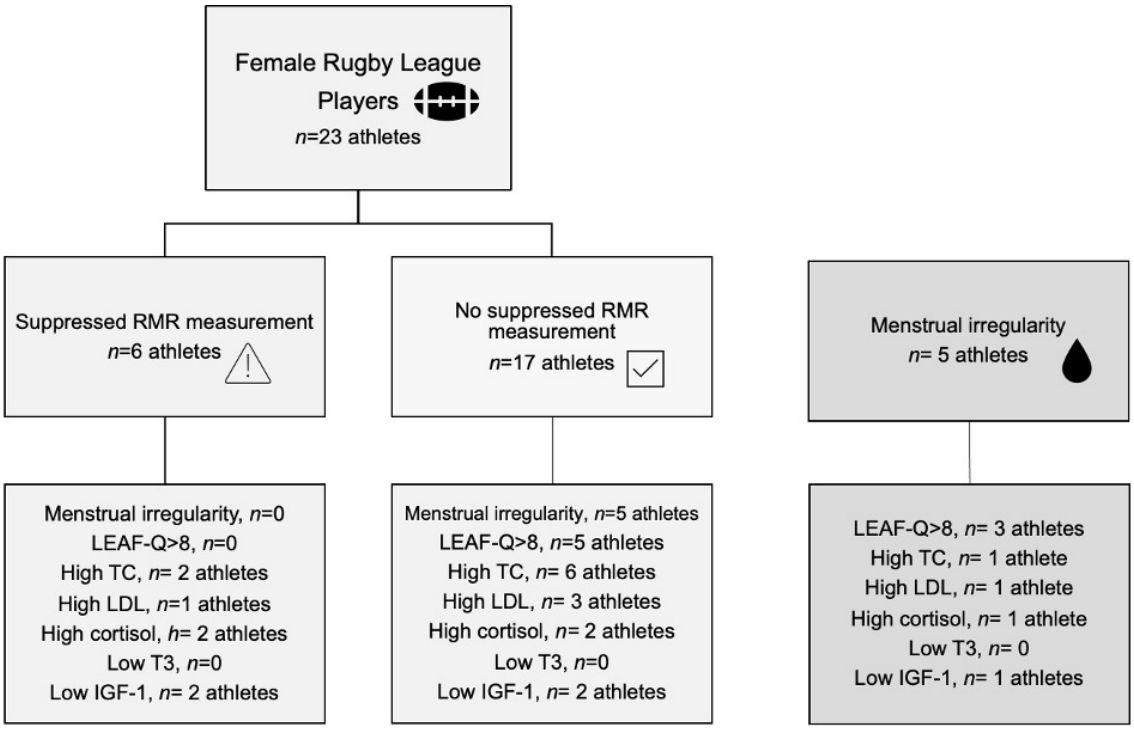

Figure 4.5. Number of athletes who presented with a suppressed RMR or menstrual irregularity across the training camp, and indicators of low energy availability within each cohort. *Note.* HC, hormonal contraceptive; NC, naturally cycling; RMR, resting metabolic rate; LEAF-Q, the Low Energy Availability in Females Questionnaire; TC, total cholesterol; LDL, low-density lipoprotein; T3, triiodothyronine.

4.5 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 standardised 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^{-1} reduction (~3%) in RMR compared to the follicular phase and ~70 kcal·day^{-1} (~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 4.4), 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 minimise factors that may contribute to technical error and variability in RMR (e.g., standardising 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 kcal·day^{-1} would require a change >60-90 kcal·day^{-1} 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 kcal·day^{-1}, with only five studies reporting an increase >60 kcal·day^{-1} 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 kcal·day^{-1} lower in the follicular compared to the luteal phase of the MC (Malo-Vintimilla et al., 2023). However, ovulation was not confirmed, and eight of the 20 participants had serum progesterone concentrations <5 ng·mL^{-1} 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 4.4). 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., 2022b). 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 (~1.0 kg, ~2.5% 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 between the early hormone phase and nonactive pill phase were below the 1.4% least significant change 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 4.3). 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 4.5), 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 a 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·kg FFM^{-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 (Heymsfield 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.

4.6 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).

Authorship

This study was designed by M.A. Kuikman, A.K.A. McKay, R. Harris, C. Minahan, and L.M. Burke. Data was analysed 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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Conflict of interest: The authors declare no conflict of interests

5 Study 1: Erratum to Effect of Menstrual Cycle Phase and Hormonal Contraceptives on Resting Metabolic Rate and Body Composition

A published erratum was made to study 1 as followed:

After publication, the authors learned there was a systematic error in the proprietary protocol to calculate resting metabolic rate (RMR) in the laboratory used for this study, resulting in systematically elevated RMR measurements. P-values and the number of athletes presenting with a suppressed RMR have been corrected in the abstract and main text; Figures 2, 4, and 5; and Table 5. The relationships between the old and new RMR calculations are linear and highly correlated, and the major findings of the study did not change. The article was corrected October 9, 2024.

The following chapter compromises the sections of the original paper as covered in chapter 4 that were corrected. The corrections made are marked in red to highlight where changes have been made. The source of the error will be elaborated on in the discussion of this thesis (chapter 9).

5.1 Abstract corrections

Original: There was no effect of MC phase on absolute RMR (p=0.877), relative RMR (p=0.957). There was no effect of HC use on absolute RMR (p=0.069), relative RMR (p=0.679).

Corrected: There was no effect of MC phase on absolute RMR (p=0.875), relative RMR (p=0.958). There was no effect of HC use on absolute RMR (p=0.068), relative RMR (p=0.309).

5.2 Results corrections

Original: 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.

Corrected: There was no effect of MC phase (p=0.875) or HC usage (p=0.068) on absolute RMR nor was there an effect of MC phase (p=0.958) or HC usage (p=0.309) on relative RMR.

Original: 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.

Corrected: There was no within athlete correlation between changes in relative RMR and estradiol concentrations (r=0.31, p=0.170); progesterone concentrations (r=0.06, p=0.786), or the ratio of concentrations of estradiol to progesterone (r=0.11, p=0.672) from Phase 1, Phase 2, and Phase 4 of the MC in NC athletes.

Original: 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). However, the RMR ratio calculated from the HB equation using ABM was greater in HC athletes than NC athletes (p=0.020).

Corrected: There was no effect of MC phase on the RMR ratio calculated from the Harris Benedict (HB) equation using actual BM (p=0.961) or ABM (p=0.149), $Cunningham_{1980}$ equation (p=0.995), or $Cunningham_{1991}$ equation (p=0.973) nor was there an effect of HC usage on the RMR ratio calculated from the HB equation using actual BM (p=0.363), $Cunningham_{1980}$ equation (p=0.975), or $Cunningham_{1991}$ equation (p=0.784). However, the RMR ratio calculated from the HB equation using ABM was greater in HC athletes than NC athletes (p=0.018).

Original: 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. No athlete presented with a RMR ratio considered suppressed when using the $Cunningham_{1980}$ or $Cunningham_{1991}$ equation or when using ABM in the HB equation.

Corrected: At the first testing measurement, four athletes presented with a suppressed RMR. However, at the second and/or third test, an additional seven athletes presented with a relative RMR <30 kcal·kg FFM^{-1}·day^{-1}. Four of these athletes also presented with a RMR ratio <0.90 using the HB equation. However, one of these athletes no longer

had a suppressed ratio when using an ABM in the HB equation. Two of these athletes also presented with a suppressed RMR using the Cunningham$_{1980}$ equation. No athlete presented with a RMR ratio considered suppressed when using the Cunningham$_{1991}$ equation or when using ABM in the HB equation.

5.3 Discussion corrections

Original: None of the athletes with menstrual irregularity during the training camp presented with a RMR measurement that met any of the criteria suggesting RMR suppression.

Corrected: Only one of the athletes with menstrual irregularity during the training camp presented with a RMR measurement that met any of the criteria suggesting RMR suppression.

Original: 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·kg FFM^{-1}·day^{-1}.

Corrected: Of the eleven athletes who did present with a suppressed RMR measurement across the training camp, six also presented with an indicator of LEA. Furthermore, for seven of the eleven athletes, this suppressed RMR was based solely on a relative RMR <30 kcal·kg FFM^{-1}·day^{-1}.

5.4 Table and figures corrections

Table 5.1. Original and corrected values for 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
Original values:				
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
Corrected values:				
Harris Benedict	1.01±0.04	1.02±0.06	1.04±0.11	1.03±0.09
Harris Benedict with ABM (*n=3 NC/5HC*)	1.05±0.06	1.04±0.02	1.07±0.07	1.14±0.03
Cuningham$_{1980}$	1.04±0.07	1.05±0.07	1.05±0.07	1.04±0.09
Cunningham$_{1991}$	1.11±0.08	1.12±0.08	1.11±0.08	1.10±0.09

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

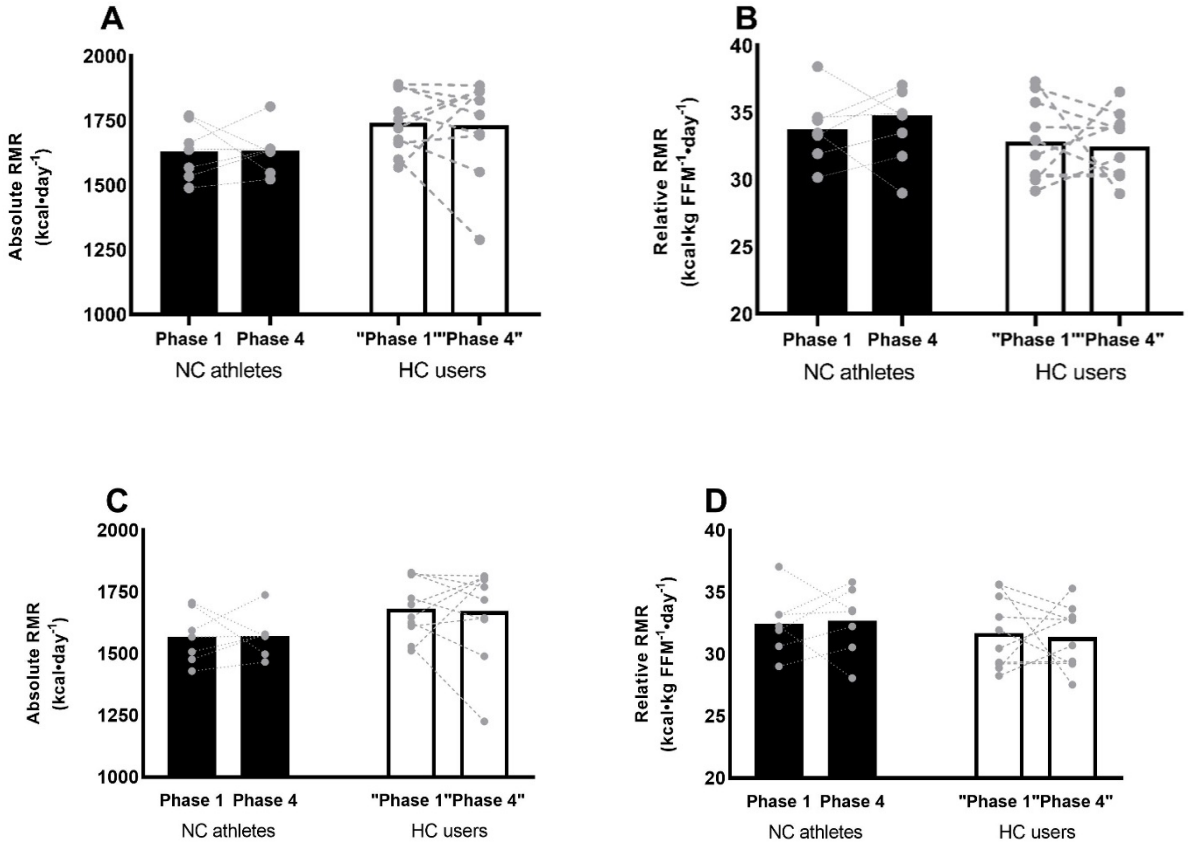

Figure 5.1. Original (from chapter 4) absolute resting metabolic rate (A), and relative resting metabolic rate (B) with menstrual cycle phase and hormonal contraceptive usage and corrected absolute resting metabolic rate (C), and relative resting metabolic rate (D). Data shown as mean with individual data points.

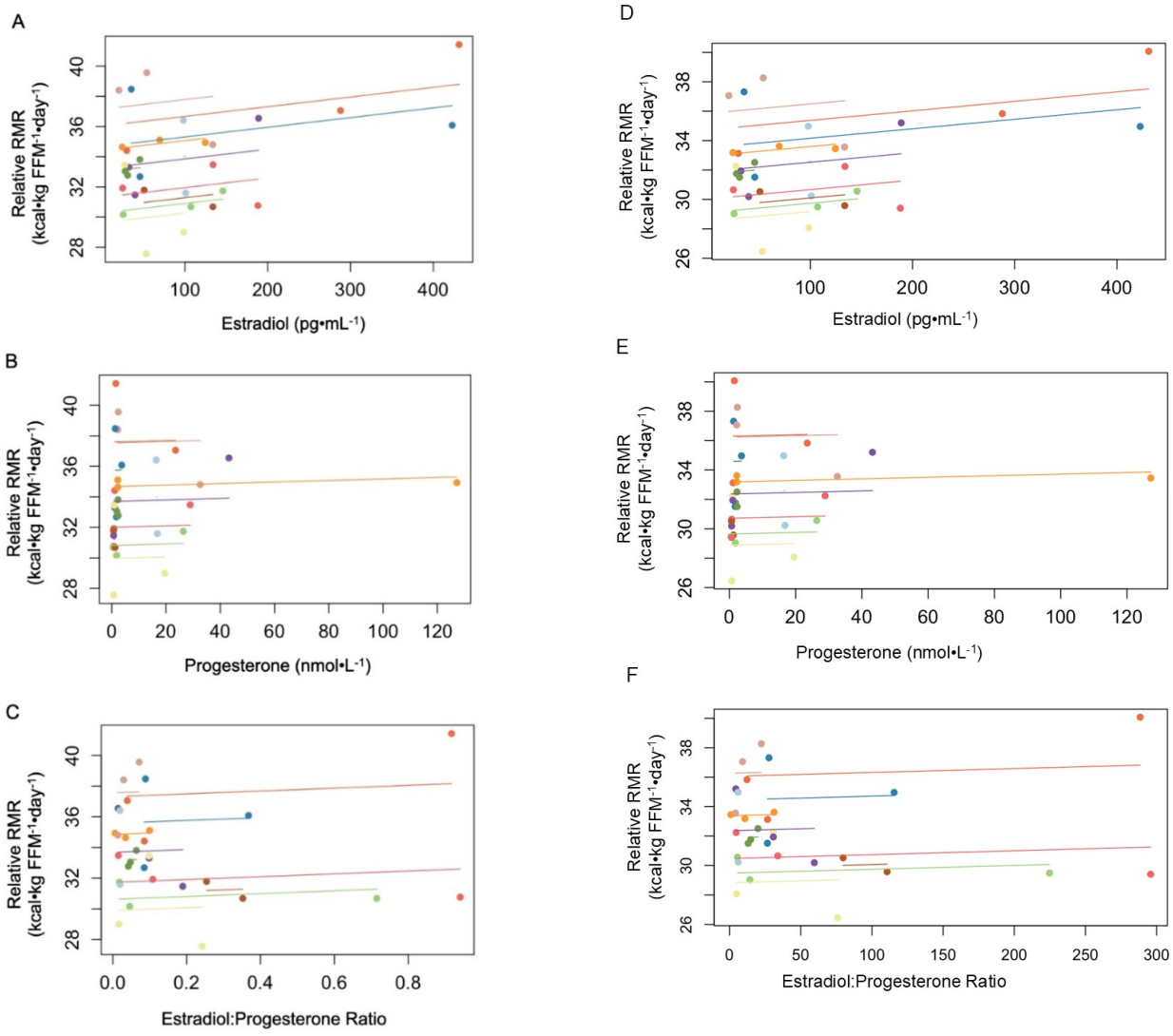

Figure 5.2. Original (from chapter 4) repeated measures correlation between relative resting metabolic rate (RMR) and serum estradiol concentrations (A), serum progesterone concentrations (B) and the concentration of estradiol to progesterone ratio (C) and corrected repeated measures correlation between relative resting metabolic rate (RMR) and serum estradiol concentrations (D), serum progesterone concentrations (E) and the concentration of estradiol to progesterone ratio (F) Separate lines fit to the data from Phase 1, Phase 2, and Phase 4 measurements for each naturally cycling athlete (n=11).

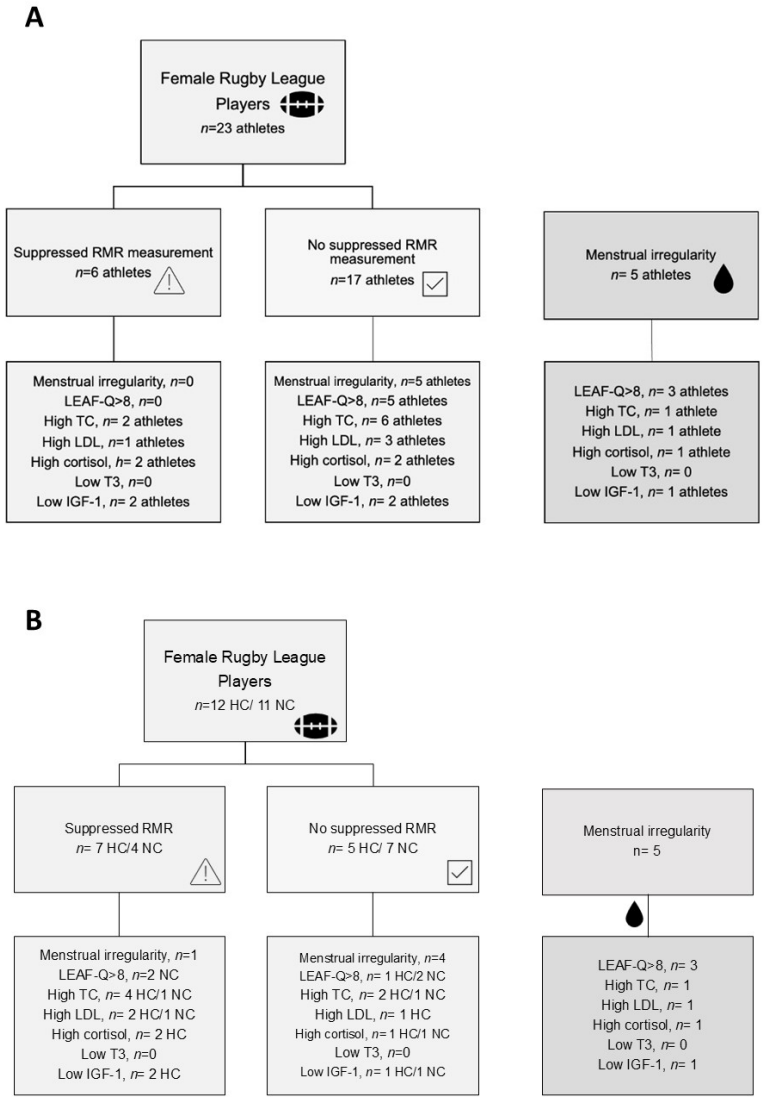

Figure 5.3. Original (from chapter 4) number of athletes who presented with a suppressed RMR or menstrual irregularity across the training camp, and indicators of low energy availability within each cohort (A) and corrected number of athletes who presented with a suppressed RMR or menstrual irregularity across the training camp, and indicators of low energy availability within each cohort (B). 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.

5.5 Interlinking chapter

The first study of this thesis demonstrated that RMR does not differ between Phase 1 and Phase 4 of the MC or with HC use in female athletes. This suggests that MC phase and HC usage does not contribute to variability in RMR measurements. As such, RMR measurements can be compared in Phase 1 or Phase 4 of the MC, or with varying HC approaches in female athletes, without variations in sex hormones causing additional noise.

The second study of thesis examined the temporal effect of altitude exposure on RMR in female athletes during a 3-week altitude training camp, and if this response is altered by LEA.

6 Study 2 The Temporal Effects of Altitude and Low Energy Availability Manipulation on Resting Metabolic Rate in Female Athletes

Publication statement:

This chapter is comprised of the following paper published in *Medicine & Science in Sports & Exercise*

Kuikman, M.A., McKay, A.K.A., McCormick, R., Tee, N., Vallance, B., Ackerman, K.E. Harris, R., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. The temporal effects of altitude and low energy availability manipulation on resting metabolic rate in female athletes. *Medicine & Science in Sports & Exercise.* 57(1), 123-133. doi: 10.1249/MSS.0000000000003534.

The chapter does not differ from the published paper apart from tables, figures and references, which have been renumbered to maintain consistency within the thesis.

6.1 Abstract

Purpose: To investigate the temporal effects of altitude exposure and energy availability (EA) manipulation on resting metabolic rate (RMR).

Methods: Twenty elite female race walkers underwent a 3-week training camp at an altitude of ~1800 m. During the first two weeks, all athletes consumed a high EA (HEA) diet of 45 kcal·kg fat free mass (FFM)$^{-1}$·day^{-1}. During the final week, half the athletes consumed a low EA (LEA) diet of 15 kcal·kg FFM^{-1}·day^{-1} while the others continued on a HEA diet. To assess the effect of altitude on RMR, athletes in the HEA group had RMR measured at baseline (~580 m) prior to altitude exposure (Pre-alt), at 36 hours (36h-alt), 2 weeks (Wk2-alt) and 3 weeks into altitude exposure (Wk3-alt), and at 36 hours post-altitude exposure at ~580 m (36h-post). To assess the effect of LEA exposure on RMR while at altitude, athletes in the LEA group underwent RMR measurements at Pre-alt as well as before (Wk2-alt) and after the 7-day period of LEA (Wk3-alt). Body composition was assessed at Pre-alt and 36h-post via Dual-energy X-ray absorptiometry.

Results: Compared to Pre-alt, RMR was increased at 36h-alt (76±40 kcal·day^{-1}, p=0.026) and Wk2-alt (70±67 kcal·day^{-1}, p=0.049), but was no longer elevated at Wk3-alt (24±64 kcal·day^{-1}; p=0.850). RMR at 36h-post was lower than all timepoints at altitude ($p<0.05$) but was not different from Pre-alt (-59±101 kcal·day^{-1}; p=0.124). The 7-day period of LEA exposure at altitude did not affect RMR (p=0.347).

Conclusion: RMR was transiently increased with altitude exposure in female athletes and was unaffected by short-term LEA. However, the altitude-induced increase was small (~25-75 kcal·day^{-1}) and was unlikely to have clinically significant implications for daily energy requirements.

6.2 Introduction

Many athletes who undertake endurance-based training include natural/terrestrial altitude (hypobaric hypoxia) training, which typically involves a 2-to-4-week period of living and training at altitudes ranging from "*low*" altitude (~500 to 2000 m) to "*moderate*" altitude (~2000 to 2500 m) (Bärtsch & Saltin, 2008; Flaherty et al., 2016). These "altitude camps" are strategically incorporated into an athlete's training and competition cycles (Mujika et al., 2019), to take advantage of the hypoxic stress and haematological and non-haematological adaptations that may result in improved performance on return to sea-level (Gore et al., 2007). While nutrition plays a key role in optimizing adaptations to altitude training (Stellingwerff et al., 2019), many issues are unstudied. Indeed, most research on the physiological response to altitude exposure includes non-athletic cohorts (e.g., military cohorts) and has been conducted at much higher altitudes (>4000 m) than the low to moderate levels (~1800-2400m) that many athletes commonly incorporate into a training cycle (Bärtsch & Saltin, 2008; Flaherty et al., 2016). A question of particular concern is whether energy requirements differ during altitude exposure due to alterations in resting metabolic rate (RMR), which represents the minimal energy cost of living (Hulbert & Else, 2004). Indeed, increases in RMR (~7-27%) have been reported upon acute altitude exposure to high altitude (~4300 m) in non-athletic men and women (Butterfield et al., 1992; Hannon & Sudman, 1973; Mawson et al., 2000). However, in women at this altitude, this increase in RMR was transient, with RMR returning to sea level values by 6-7 days of altitude exposure (Hannon & Sudman, 1973; Mawson et al., 2000). At a more moderate altitude (~2200 m), an increase in the RMR (~19%; ~290 kcal·day^{-1}) of male and female middle-distance runners was detected at the end of a 4-week altitude training camp (Woods, Sharma, et al., 2017). However, given the small sample size (3M/2F), this study may have been underpowered (Woods, Sharma, et al., 2017). Furthermore, it is possible that an even greater increase in RMR occurred with acute altitude exposure in this cohort of athletes, as has

previously been seen at higher altitudes (Butterfield et al., 1992; Hannon & Sudman, 1973; Mawson et al., 2000), but RMR was measured only at baseline and the camp's end and did not investigate the acute response to hypoxia (Woods, Sharma, et al., 2017). Determining any increases in basal energy requirements associated with altitude exposure is important when considering nutritional support of athletes.

Despite the potential for energy requirements to be increased with altitude exposure due to alterations in RMR, athletes may purposefully restrict energy intake (EI) during altitude training camps due to a desire to alter body composition or may inadvertently fail to consume sufficient energy due to changes in appetite (Debevec, 2017). Reduced food availability in a new environment or increases in training load during altitude training camps may further perpetuate inadequate EI with altitude exposure. Increased energy requirements and/or reduced EI may result in a state of low energy availability (LEA) with energy availability (EA) representing the dietary energy remaining to support the body's health and physiological basal functioning after exercise energy expenditure (EEE) has been subtracted (Loucks et al., 2011). LEA exposure that results in persistent disruptions in body systems can lead to signs and symptoms of Relative Energy Deficiency in Sport (REDs) (Mountjoy et al., 2023). A case study involving elite male and female rowers observed a trend for reduced RMR (~5%) and loss of fat mass on return from a 12-day training camp at altitude (~1800 m) (Woods, Garvican-Lewis, et al., 2017). This was attributed to LEA exposure in the absence of a controlled EI during the camp (Woods, Garvican-Lewis, et al., 2017) as LEA exposure leads to a decreased RMR (Mountjoy et al., 2023). Yet, failure to measure RMR at altitude prevents the ability to discern the effects of altitude versus LEA exposure. This highlights the importance of controlling EA when measuring changes in RMR to ensure that LEA is not confounding results. However, examining the effect of LEA exposure on RMR while at altitude is also necessary to

understand how bodily systems are differently affected by LEA exposure and the contribution of altitude exposure as a moderating factor (Burke et al., 2023). For instance, concurrent increases in RMR from altitude exposure may be neutralised by LEA exposure, causing minimal overall effect on net changes in RMR.

In order to better understand an athlete's energy requirements during altitude training camps, it is necessary to determine if RMR is altered with altitude exposure and the time course of such changes. Furthermore, determining if LEA alters this response is needed to better understand the specific effects of LEA exposure and moderating factors on REDs outcomes. As such, the purpose of this study was to investigate the temporal effects of altitude exposure and LEA manipulation on RMR in female athletes.

6.3 Methods

6.3.1 Participants

Twenty female race-walkers (26.5±6.5 years, VO_2max: 58.2±4.2 $ml \cdot kg^{-1} \cdot min^{-1}$) of Tier 3 (Highly Trained/National Level) to Tier 5 (World Class) calibre (McKay et al., 2022) were recruited for this study. Naturally menstruating (defined as non-hormonal contraceptive using athletes with self-reported cycle lengths between 21 and 35 days; NM) athletes (n=13) and hormonal contraceptive (HC) users (n=6 oral contraceptive pill (OCP), n=1 Implanon) were recruited. The oral contraceptive pill (OCP) used by HC users included both combined (n=1 Optilova, n=1 Bellaface suave, n=1 Harmonet, n=1 Evaluna20, n=1 Zoely) and progesterone only (n=1 Slinda). Details on OCP preparation can be found as supplementary material (supplementary table 1) on the journal website. It was not possible to standardise menstrual cycle or HC phase within RMR measurements because the research-embedded training camp study design required that all athletes needed to travel to altitude and begin the study at the same time. Additionally, noting that the elite calibre of athletes in this study represents

~0.014% of the global population (McKay et al., 2022), it was not feasible to only include athletes of homogenous menstrual status (i.e., only NM athletes or HC users using one brand of OCP) as this would severely limit the sample size. Nevertheless, the potential influence of reproductive hormones in this study is likely small, given that we have previously shown that RMR appears to be unaffected by menstrual cycle phase and HC usage in athletic cohorts (Kuikman, McKay, et al., 2024). As such, the ovarian hormone profiles were provided to describe the menstrual characteristics of athletes rather than to the control the hormonal profile and examine the effects of hormones on research outcomes. The menstrual status (MS) of each athlete was characterised twice (i.e., upon recruitment via self-reported means and at the end of the study when MS could be retrospectively verified via measured outcomes) with consideration of the Best Practice Guidelines (Elliott-Sale et al., 2021). At recruitment all NM athletes reported ≥9 periods in the preceding year. Thereafter they tracked their menstrual cycle from 4 weeks preceding the study until 1 week after study completion using an online reporting system (REDCap), and tested for ovulation beginning on day 8 of the menstrual cycle using urinary luteinising hormone (LH) surge testing (Advanced Digital Ovulation Test, Clearblue, Geneva, Switzerland). HC users reported bleeding using the same online reporting system. In addition, hormonal profiles of estradiol and progesterone were established at three time points throughout the training camp (pre-altitude exposure, at 2 weeks altitude exposure and post-altitude exposure) for both NM athletes and HC users. Data of one NM athlete were excluded from analysis due to an injury sustained during the first week at altitude, thus preventing full completion of the study. Athletes were informed of the risks and requirements of the study before providing informed consent. Ethics approval was obtained from the Ethics Committee at Australian Catholic University.

6.3.2 Experimental design

Baseline testing occurred at the Australian Institute of Sport (AIS) in Canberra, Australia (~580 m) over a 5-day period during which time all athletes had standardised dietary control. Athletes then travelled by vehicle to Perisher Valley, Australia (~1800 m) for a 3-week altitude training camp before returning to Canberra for post-altitude testing that occurred over a 4 day period (See Figure 6.1). The first 2 weeks at altitude served as an acclimatisation period during which all athletes consumed a fully provided diet providing an EA of 45 kcal·kg FFM^{-1}·day^{-1}. This was followed by a 7-day dietary intervention, which manipulated EA. During this dietary intervention, one group of athletes (n=10) consumed a diet providing an EA of 15 kcal·kg FFM^{-1}·day^{-1} (LEA) while the remaining athletes (n=9) continued to consume a diet providing an EA 45 kcal·kg FFM^{-1}·day^{-1} (high energy availability; HEA). Athletes were allocated into groups based on individual preferences for the EA intervention, with athletes who nominated no preference allocated strategically to ensure key characteristics (e.g., menstrual status, athlete calibre, etc.) were balanced between dietary groups.

In order to assess the time course of potential changes in RMR at altitude, athletes in the HEA group had RMR measured pre-altitude exposure during the baseline testing period (Pre-alt), after ~36 hours exposure to altitude (36h-alt), 2 weeks altitude exposure (Wk2-alt), 3 weeks altitude exposure (Wk3-alt), and ~36 hours post-altitude (36h-post). To assess the impact of LEA on RMR measurements, athletes in the LEA group had RMR measured at Pre-alt, and before and after the dietary intervention, which corresponded to a RMR measurement at Wk2-alt and Wk3-alt. Body composition was also assessed using Dual-energy X-ray absorptiometry (DXA) at Pre-alt and 36h-post.

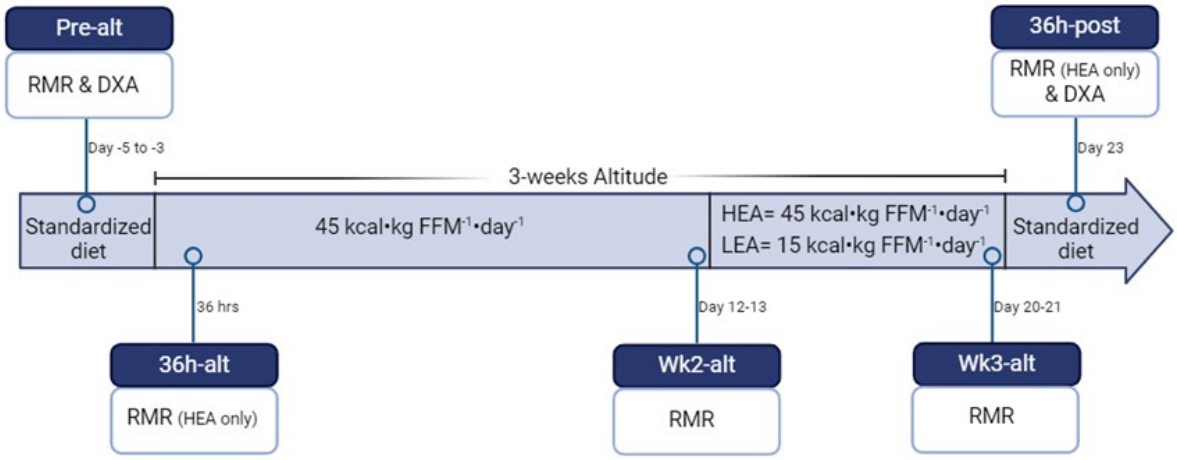

Figure 6.1. Schematic diagram of study design, detailing elevation, timeline, dietary protocols, and measurements taken. FFM, fat free mass; RMR, resting metabolic rate; DXA, dual-energy-x ray absorptiometry; HEA, high energy availability; LEA, low energy availability.

6.3.3 Dietary intervention

For 4 days before and 3 days after the altitude training camp, all participants consumed a standardised diet that provided ~8 g·kg $^{-1}$ carbohydrate, ~1.5 g·kg^{-1} protein and ~1.1 g·kg^{-1} fat, resulting daily energy intake of ~48 kcal·kg^{-1}. During the altitude training camp, daily energy requirements were determined prospectively for each athlete based on individualised training plans and calculated using the following equation: EI = (Target EA × FFM) + EEE. Daily protein intake was the same for both dietary interventions and provided ~2 g·kg^{-1}. When receiving a diet that contained an EA of ~45 kcal·kg FFM^{-1}·day^{-1}, ~20% of energy intake was from fat whereas the LEA diet provided ~15% of energy intake from fat. Regardless of the target EA, the remaining energy came from carbohydrates. Individual meal plans were created for each athlete based on planned training for that day and personal preference, with a chef preparing all meals.

Training load (volume x intensity) was not controlled throughout the altitude training camp. Rather, athletes followed their individualised training plans throughout the duration of the

study. Daily EEE was prospectively estimated from an athlete's planned training, which included race walking, running, cycling, and/or resistance training across 1-3 sessions·day^{-1}. The EEE of a race-walking training session was determined from the individualised gas exchange data collected during a 4-stage submaximal race-walking graded exercise test (GXT) completed on a treadmill during the Pre-alt period at the AIS. EEE during each GXT stage was determined using the Weir equation with Pre-alt RMR excluded from the same period as follows: [(3.94 × VO_2 + 1.11 × VCO_2) – (24 hr RMR/1440 (min))] (Weir, 1949). EEE per km of outdoor race walk training was then estimated from each speed of the GXT as follows: (($EEE_{kcal/min}$ × 60 min))/$Speed_{km/hr}$). Walking EEE ranged from 0.88-1.07 kcal·km^{-1}·kg^{-1} (average ~1 kcal·km^{-1}·kg^{-1}). Running EEE was estimated as kilometre ran multiplied by an athlete's body mass (1 kcal·km^{-1}·kg^{-1}) (Margaria et al., 1963), cycling using a Metabolic Equivalent (MET) of 8, and resistance training a MET of 4 (Jetté et al., 1990). Pre-alt RMR was again excluded from the same time-period when estimating EEE for running, cycling and/or resistance training sessions.

Athletes reported their actual training daily to a member of the research team and EI was adjusted if the difference in EEE between actual training and planned training exceeded the EEE of 2 km of race walking. When increases in EI were needed, this was accomplished by increasing portion sizes at meals and/or providing additional snacks. When decreases in EI were needed, this was accomplished by decreasing the portion size of the day's final meal and/or removing snacks. Two days of ad libitum food intake were scheduled within the training camp: the day of ascent to altitude (day 1) and the day prior to commencing the 7-day dietary intervention after undergoing the Wk2-alt RMR measurement (day 13). These were implemented for logistical reasons and to provide participants a break from dietary control given the extensive nature and dietary compliance that this study involved.

6.3.4 Measurements

6.3.4.1 Body composition

DXA scans were done in accordance with Best Practice Guidelines (Slater, Townsend, et al., 2023) before and after the altitude training camp. Athletes presented for testing in an overnight fasted state and with no fluid intake prior to the scan. All scans were conducted by the same researcher with consistent positioning of participants on the DXA scanning bed using Velcro straps and positioning aids. Scans were performed in the same mode (GE Lunar iDXA) and analysed using GE encore, which provided an assessment of FFM, lean body mass (LBM), and fat mass.

6.3.4.2 Resting metabolic rate

RMR was measured using the ParvoMedics TrueOne 2400 metabolic cart (ParvoMedics, Salt Lakes City UT, USA). The ParvoMedics system was calibrated with gas concentrations (15.99% O_2, 4.00% CO_2) and ventilation using a 3L syringe prior to testing. Athletes presented after an overnight fast and before morning training around the same time of day (± 30 minutes) to account for circadian changes in RMR (Zitting et al., 2018). At the AIS, athletes resided in a residence building next to where the RMR measurements occurred and while at altitude, RMR measurements occurred in the lodge where athletes resided. As such, upon waking, athletes were only required to walk a short distance to where the RMR measurement occurred. Upon arrival, athletes laid in a supine position in a dark, quiet room for 10 minutes to ensure a state of rest and were then given a one-way mouthpiece that was connected to the ParvoMedics cart for a 10-minute familiarisation period. Expired air was then collected for a single 25-minute period. Upon completion, data were exported into a Microsoft excel file. The first 2 minutes and last 2 minutes of each 25-minute period were discarded and a mean was calculated from the remaining minutes to estimate a 24-hour absolute RMR ($kcal \cdot day^{-1}$) using the Weir equation (Weir, 1949).

6.3.4.3 Indicators of low EA

Indicators of LEA were measured throughout the training camp (Stellingwerff et al., 2023). Primary indicators included: triiodothyronine (T3) concentrations. Secondary indicators included low density lipoprotein (LDL) and total cholesterol (TC) concentrations (Stellingwerff et al., 2023). Potential and emerging indicators included: insulin like growth factor one (IGF-1) concentrations, cortisol concentrations, and RMR (Stellingwerff et al., 2023). RMR measurements were used to assess for a suppressed RMR by calculating a RMR ratio (measured RMR:predicted RMR) using the Cunningham 1990, Cunningham 1991, and Harris benedict (HB) equations to predict RMR (Cunningham, 1991, 1980; Harris & Benedict, 1918) as well as relative RMR (measured RMR:FFM). These were selected given they have validated thresholds with a suppressed RMR being defined as a RMR ratio <0.90 when using the Cunningham 1980 or HB equation, RMR ratio <0.92 when using the Cunningham 1991 equation (Strock et al., 2020) and/or a relative RMR <30 kcal·kg FFM^{-1}·day^{-1} (Loucks et al., 2011). Athletes were not assessed as per the updated REDs Clinical Assessment Tool V.2 (REDs CAT2) (Stellingwerff et al., 2023) to ascertain their risk of REDs because the study was undertaken prior to its publication and did not capture data on all primary risk factors, increasing the risk of a false negative assessment.

6.3.4.4 Blood samples

An 8.5 mL venous blood sample was collected from an antecubital vein into a serum separator tube by a trained phlebotomist at Pre-alt, Wk2-alt and 36h-post. Blood tubes were left to clot at room temperature for 30 minutes, prior to centrifugation at 1500 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). Intra-assay coefficient of variations were 5% for estradiol and 11%

for progesterone. Lipids, cortisol, IGF-1, and T3 were measured by chemiluminescent immunoassay through a commercial laboratory (Laverty Pathology, Bruce, ACT, Australia).

6.3.5 Statistics

Statistical analyses were performed using R Studio (v3.5.2) with statistical significance accepted at an α level of $p \leq 0.05$. The insulin results of two athletes (n=1 LEA athlete, n=1 HEA athlete) were considered outliers due to values being >3 SD above the mean and excluded from analyses. Histogram inspection revealed non-normally distributed data for fat mass, which were then log transformed for analyses. Statistical analyses were completed using general linear mixed models where significance of fixed effects was tested using type II Wald F tests with Kenward-Roger degrees of freedom. For statistical analyses of RMR measurements, two separate models were used. One model assessed time course change in the HEA group only, which included test time point (Pre-alt, 36h-alt, Wk2-alt, Wk3-alt, 36h-post) as a fixed effect and subject as a random effect. The other model assessed the effect of EA manipulation, which included test time point (Pre-alt, Wk2-alt, Wk3-alt) and dietary intervention (HEA or LEA group) as a fixed effect. With this model, subject and body mass were used as a random effect except for the model assessing relative RMR which only had subject as a random effect. For the models assessing diet, body composition, and LEA indicators, test time point, and dietary intervention were fixed effects and subject was a random effect. For models assessing cortisol and T3, body mass was also included as a random effect. Where significant effects were evident, a Tukey's post-hoc comparison was performed.

6.4 Results

6.4.1 Dietary analysis

As intended, energy and macronutrient intake during the standardised diet period did not differ between athletes in the HEA and LEA group or between the Pre-alt and 36h-post period (all p>0.05, see supplementary table 2 on the journal website). Table 6.1 outlines the EEE, EI, EA,

and macronutrient intake during the acclimatisation and dietary intervention period at altitude. Daily EEE was greater during the dietary intervention period compared to the acclimatisation period ($p<0.001$), and the EEE of athletes in the LEA group was higher than that of athletes in the HEA group ($p<0.001$), however no interaction was evident ($p=0.779$). As intended, the EA, energy, carbohydrate, and fat intake was lower for athletes in the LEA group during the dietary intervention compared to their intake during acclimatisation period and compared to athletes in the HEA group during both the acclimatisation period and dietary intervention period ($p<0.0001$). The protein intake did not differ between athletes in the HEA and LEA group ($p=0.659$), but protein intake during the acclimatisation period was marginally higher (+0.1 $g \cdot kg^{-1} \cdot day^{-1}$) compared to the dietary intervention period for both groups ($p<0.0001$).

Table 6.1. Mean daily training, exercise energy expenditure, and dietary intake during the 12-day acclimatisation period and 7-day dietary intervention at altitude. Data presented as mean ± standard deviation. *Significant compared to acclimatisation period and HEA during the dietary intervention period. #Significant compared to LEA during the acclimatisation period. HEA, high energy availability; LEA, low energy availability; EA, energy availability; EEE, exercise energy expenditure; CHO, carbohydrate.

	Acclimatisation		Dietary intervention		P-value		
	HEA	LEA	HEA	LEA	Week	Intervention	Interaction
Race walk (km)	12.9±2.8	15.1±2.2	15.0±2.3	16.7±2.9	0.012	0.032	0.700
Run (km)	1.4±1.1	2.4±1.3	1.0±2.1	1.8±1.4	0.160	0.104	0.727
Weights (min)	13.6±9.4	14.4±6.1	7.2±5.9	10.7±7.4	0.002	0.469	0.407
Cross training (min)	5.3±8.2	2.2±3.7	4.4±7.4	1.5±4.7	0.671	0.154	0.940
EEE (kcal)	824±112	983±93	915±135	1062±11	<0.001	<0.001	0.779
EI (kcal)	2764±260	3018±159	2811±350	1732±119*	<0.0001	<0.0001	<0.0001
EA (kcal·kg FFM^{-1})	46.2±0.6	45.9±0.5	45.1±1.0	15.1±0.6*	<0.0001	<0.0001	<0.0001
CHO ($g \cdot kg^{-1}$)	8.3±0.7	9.0±0.5	8.5±0.7	4.6±0.4*	<0.0001	<0.0001	<0.0001
Protein ($g \cdot kg^{-1}$)	2.1±0.1	2.1±0.1	2.0±0.04	2.0±0.03	<0.0001	0.659	0.293
Fat ($g \cdot kg^{-1}$)	1.2±0.1	1.3±0.1	1.2±0.1#	0.6±0.1*	<0.0001	<0.0001	<0.0001

6.4.2 Menstrual status

For OCP users, only one athlete had testing during a placebo pill day of the OCP cycle with the remaining testing occurring during the active pill days. For the single athlete with an implant, all testing occurred on days without bleeding. In accordance with Best Practice Guidelines (Elliott-Sale et al., 2021), detailed information on the MC characteristics can be found as supplementary material (supplementary table 3) on the journal website. Individual estradiol, progesterone levels and the corresponding ratio at Pre-alt, Wk2-alt and 36h-post can be found as supplementary material (supplementary table 4) on the journal website.

6.4.3 Body composition

Body composition across the altitude training camp is summarised in Table 6.2. Athletes in the LEA group ($p<0.001$), but not the HEA group ($p=0.250$) had a reduction in body mass from Pre-alt to 36h-post. For athletes in both groups, FFM ($p=0.408$) and LBM ($p=0.421$) did not change, but fat mass decreased ($p<0.0001$) from Pre-alt to 36h-post.

Table 6.2. Body composition before and after the 3-week altitude training camp for athletes in the HEA and LEA group. Data presented as mean ± standard deviation. *Significant compared to pre-alt. HEA, high energy availability; LEA, low energy availability; BM, body mass; FFM, fat free mass; LBM, lean body mass; FM, fat mass.

	Pre-alt		Post-alt		Change		P-values		
	HEA	LEA	HEA	LEA	HEA	LEA	Visit	Intervention	Interaction
BM (kg)	52.9±6.0	54.8±5.0	52.6±5.8	53.9±5.1*	-0.35±0.61	-0.89±0.47	<0.0001	0.532	0.030
FFM (kg)	41.8±4.8	44.3±2.9	42.0±4.7	44.4±2.9	0.16±0.65	0.12±0.81	0.408	0.160	0.909
LBM (kg)	39.5±4.6	42.0±2.9	39.7±4.5	42.1±2.8	0.16±0.66	0.12±0.81	0.421	0.155	0.901
FM (kg)	11.1±2.4	10.4±3.5	10.6±2.4*	9.4±3.3*	-0.54±0.57	-1.00±0.60	<0.0001	0.354	0.053

6.4.4 RMR with altitude exposure

Absolute RMR was increased from Pre-alt to 36h-alt (+5.3±3.1%; p=0.026) and Wk2-alt (+4.9±4.9%; p=0.049), but was no longer elevated by Wk3-alt (+1.7±4.2%; p=0.850) or 36h-post (-3.9±7.2%; p=0.124; Figure 6.2). Absolute RMR at 36h-post was decreased compared to measurements taken at 36h-alt (-10.0±7.1%; p<0.0001), Wk2-alt (-9.4±5.3%; p=0.0001) and Wk3-alt (-6.1±6.0%; p=0.012). Changes in relative RMR followed the same trends with increased values at 36h-alt (+5.3±3.1%; p=0.016) and Wk2-alt (+4.9±4.9%; p=0.034) compared to Pre-alt, but no longer elevated at Wk3-alt (1.2±3.5%; p=0.931). Relative RMR at 36h-post was decreased compared to all values at altitude (all p<0.01), and there was a trend for a decrease in relative RMR at 36h-post compared to Pre-alt (-4.3±6.9%; p=0.052).

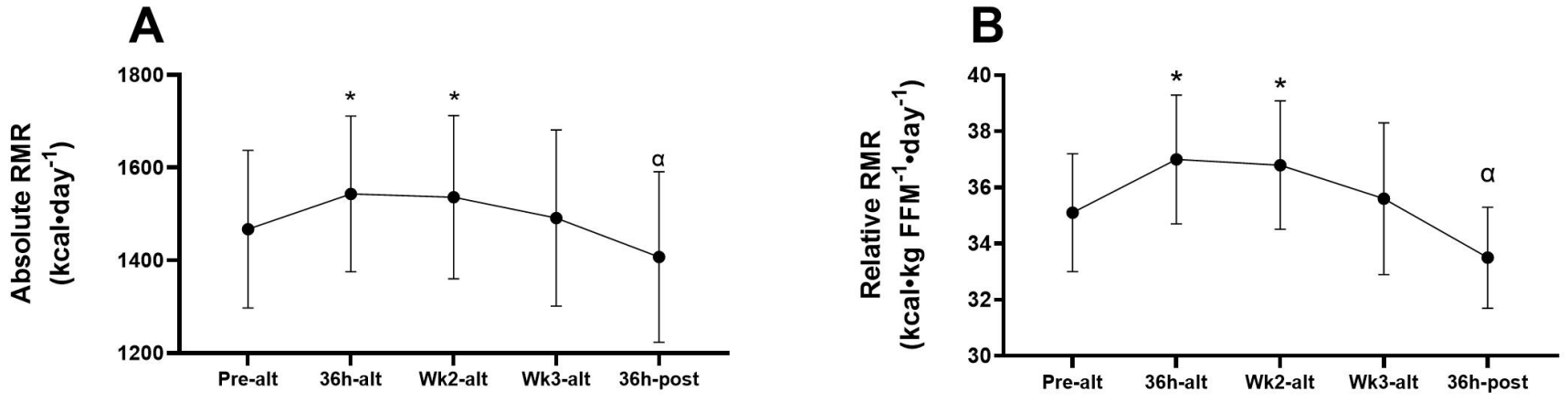

Figure 6.2. Absolute resting metabolic rate (A) and relative resting metabolic rate (B) at baseline (Pre-alt), 36 hours altitude exposure (36h-alt), 2 weeks altitude exposure (Wk2-alt), 3 weeks altitude exposure (Wk3-alt), and 36 hours post-altitude (36h-post). Data are presented as mean ± standard deviation *Different compared to Pre-alt, $^{\alpha}$Different compared to all measurements at altitude. RMR, resting metabolic rate; FFM, fat free mass.

6.4.5 RMR with LEA Exposure

The 7 days of LEA exposure at altitude did not affect absolute RMR (p=0.347) or relative RMR (p=0.547) (Figure 6.3). Two of the ten athletes in the LEA group had a decrease in RMR that exceeded 60 kcal (>4% variation in baseline RMR) from Wk2-alt to Wk3-alt. Greater inter-individual variation was noted in the HEA group with five of the nine athletes having a decrease in RMR >60 kcal from Wk2-alt to Wk3-alt.

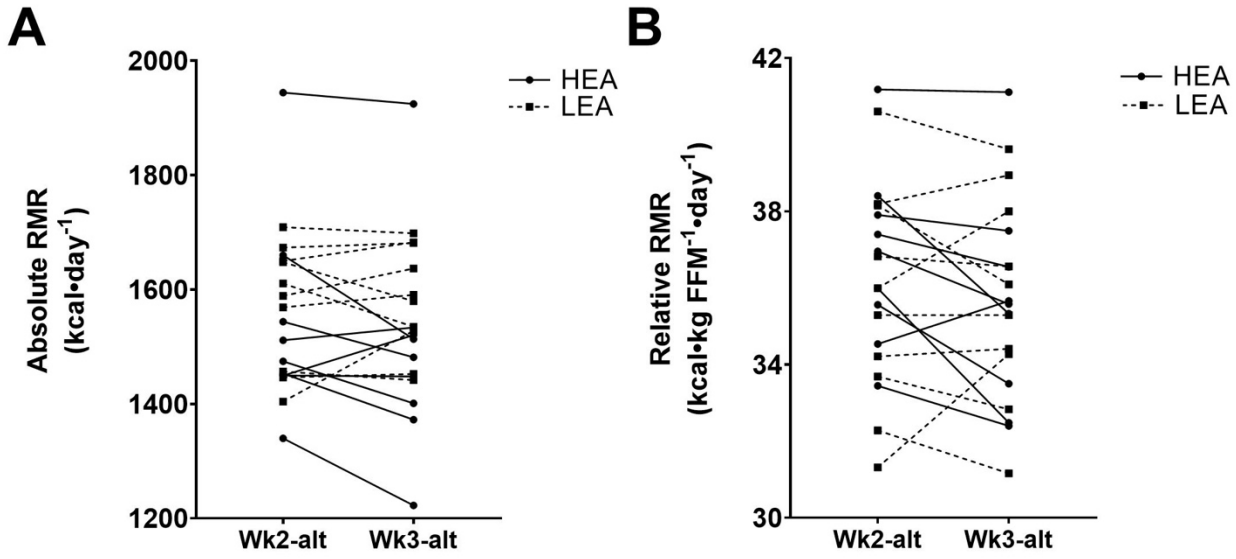

Figure 6.3. Absolute RMR (A) and relative RMR (B) before (Wk2-alt) and after (Wk3-alt) the 7 day dietary intervention for athletes in the HEA group and LEA group. Each line represents an individual athlete.

Given the unexpected change in body composition, we reanalysed changes in RMR while at altitude between athletes who did (n= 5 HEA + n=7 LEA) and did not (n= 4 HEA + n =3 LEA) have a decrease in fat mass over the training camp that exceeded the least significant change (LSC) of 4.7% (Slater, Farley, et al., 2023) regardless of dietary intervention allocation. Like LEA exposure, we found no effect of fat mass reduction (p=0.282) on changes in RMR at altitude (Figure 6.4).

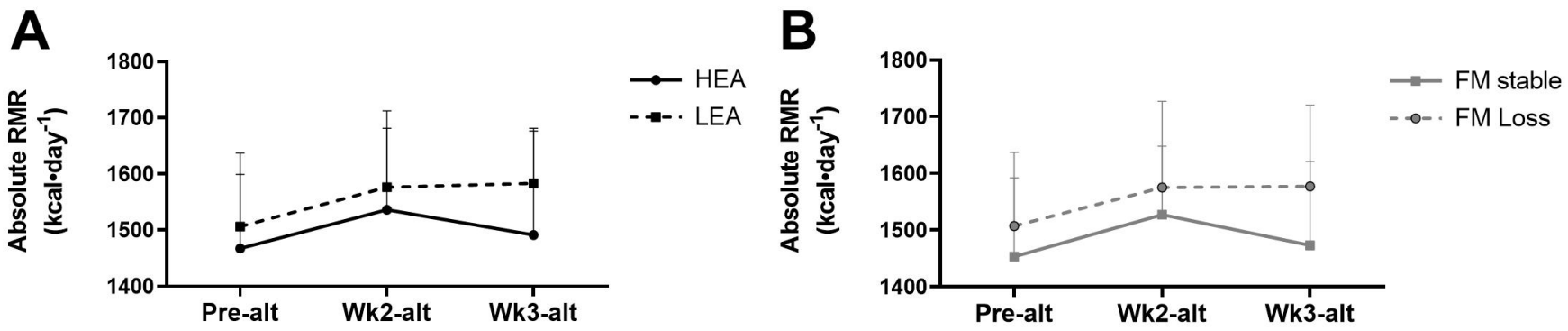

Figure 6.4. Absolute RMR at baseline (Pre-alt), 2 weeks altitude exposure (Wk2-alt) and 3 weeks altitude exposure (Wk3-alt) for athletes in the HEA group and LEA group (A) and for athletes that had a reduction in fat mass (n=5 HEA, 7LEA) or no change in fat mass (n= 4 HEA, 3 LEA) across the training camp (B). Data are presented as mean + standard deviation.

To explore the inter-individual variation for changes in RMR during the final week at altitude, a Pearson correlation was used to assess the association between change in RMR from Wk2-alt to Wk3-alt and changes in determinants of RMR across the altitude training camp. There was a negative correlation between change in RMR from Wk2-alt to Wk3-alt and change in fat mass over the training camp for athletes in the HEA group ($r=-0.735$; $p=0.024$), but not for athletes in the LEA group ($r=0.102$; $p=0.778$). No correlation was seen for change in RMR from Wk2-alt to Wk3-alt and change FFM over the training camp for athletes in the HEA group ($r=0.583$; $p=0.099$) or athletes in the LEA group ($r=-0.081$; $p=0.823$). There was also no correlation for change in RMR from Wk2-alt to Wk3-alt and change in T3 concentrations over the training camp for athletes in the HEA group ($r=0.145$; $p=0.710$), or for athletes in the LEA group ($r=-0.367$; $p=0.297$).

6.4.6 Indicators of LEA

No athlete had a RMR measurement that was considered suppressed over the course of the study using RMR ratio or relative RMR thresholds. The RMR ratio (using each predictive equation) was increased at 36h-alt ($p<0.03$) and Wk2-alt ($p<0.05$) compared to Pre-alt, but was no longer increased at Wk3-alt ($p>0.05$) or 36h-post ($p>0.05$) (Figure 6.5). The RMR ratio at 36h-post was lower than all RMR ratios at altitude (all $p<0.01$). The 7 days of LEA exposure did not affect the RMR ratio calculated from the HB ($p=0.286$), Cunningham 1980 ($p=0.868$), or Cunningham 1991 equations ($p=0.953$).

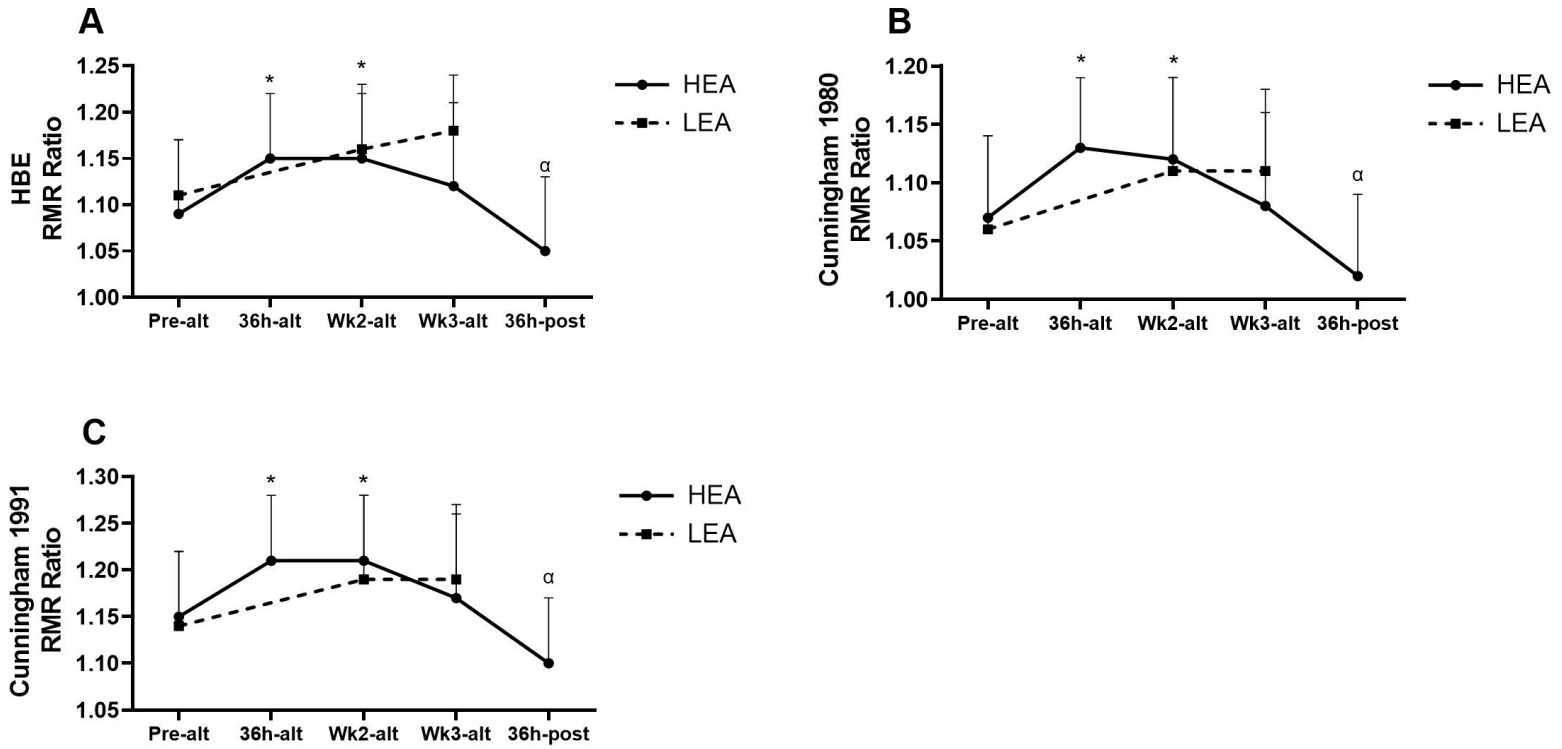

Figure 6.5. Resting metabolic rate ratio with the Harris Benedict equation (A), Cunningham 1980 equation (B) and Cunning 1991 equation (C) at baseline (Pre-alt), 36 hours altitude exposure (36h-alt), 2 weeks altitude exposure (Wk2-alt), 3 weeks altitude exposure (Wk3-alt), and 36 hours post-altitude (36h-post). Data are presented as mean + standard deviation. *Different compared to Pre-alt, αDifferent compared to all measurements at altitude. RMR, resting metabolic rate; HEA, high energy availability; LEA, low energy availability; HBE, Harris Benedict equation.

In the LEA group, T3 concentrations were lower at 36h-post compared to both Pre-alt ($p=0.002$) and Wk2-alt ($p=0.025$); cortisol concentrations were greater at Wk2-alt ($p<0.0001$) and 36h-post ($p<0.001$) compared to Pre-alt (Figure 6.6). LEA and HEA groups both had lower TC concentrations at Wk2-alt compared to Pre-alt ($p=0.041$). While there was an interactive effect of LDL ($p=0.001$), IGF-1 ($p=0.015$), and insulin ($p=0.036$), post-hoc testing was non-significant ($p>0.05$). There was a trend for differences in LDL between Wk2-alt and 36h-post for athletes in the HEA group ($p=0.05$).

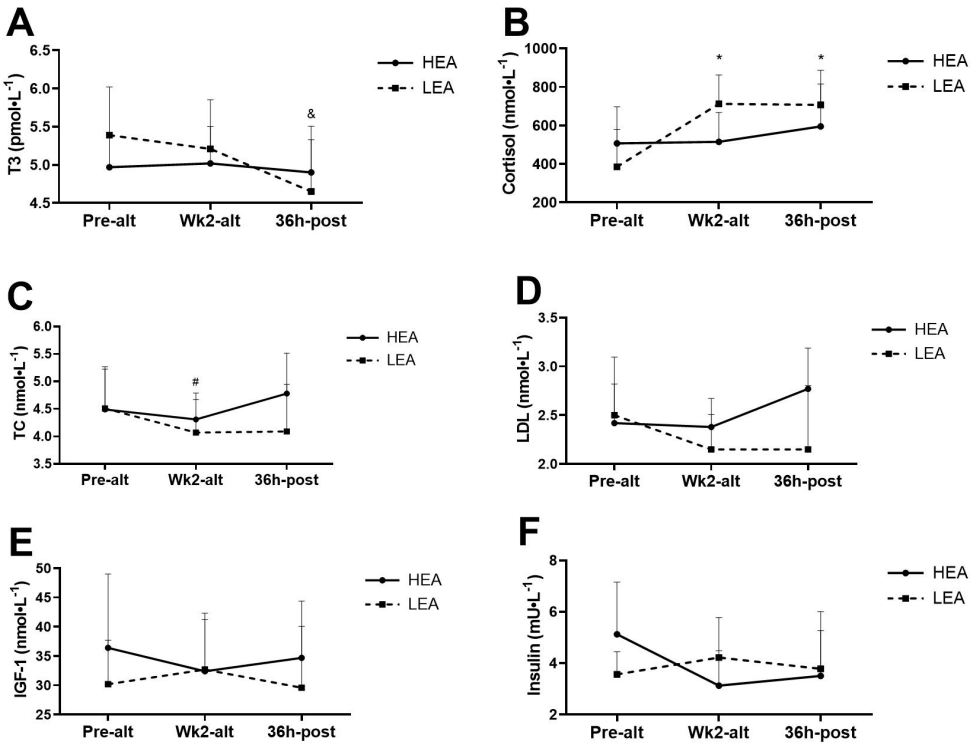

Figure 6.6. Triiodothyronine (A), cortisol (B), total cholesterol (C), low density lipoprotein (D) insulin like growth factor 1 (E) and insulin (F) levels at baseline (Pre-alt), 2 weeks altitude exposure (Wk2-alt), and 36 hours post-altitude (36h-post). Data are presented as mean + standard deviation. [&]Different compared to Pre-alt and Wk2-alt alt for athletes in the LEA group, *Different compared to Pre-alt for athletes in the LEA group, [#]Different compared Pre-alt for both groups. T3, triiodothyronine; TC, total cholesterol; LDL, low density lipoprotein; IGF-1, insulin-like growth factor one; HEA, high energy availability; LEA, low energy availability.

6.5 Discussion

The main finding of this study, implemented as a research-embedded training camp, was a transient increase in RMR with exposure to ~1800 m altitude but no change in RMR in association with a 7-day period of LEA at this altitude. The increase in RMR (~5.3% or ~75 kcal·day^{-1}) was greatest with acute (36 hour) exposure, but differences across 3 weeks of altitude exposure were not significant (~1.7% or ~24 kcal·day^{-1}). These findings are novel and build on previous athlete research pertaining to RMR changes with altitude exposure (Woods, Garvican-Lewis, et al., 2017; Woods, Sharma, et al., 2017), as we examined a time course for RMR change at altitude, and also investigated if EA alters this response.

6.5.1 RMR with altitude exposure

Our observed ~2-5% increase in RMR was smaller than the ~19% increase in RMR previously reported in highly trained middle-distance runners (n=3 males/2 females) at the end of a 4-week altitude training camp at ~2200 m, where baseline measures also occurred at ~580 m (Woods, Sharma, et al., 2017). The smaller RMR increase that we observed may be due to a smaller elevation increase between the studies (1220 m vs.1620 m) (Woods, Sharma, et al., 2017). However, the ~19% increase reported at ~2200 m (Woods, Sharma, et al., 2017) is greater than the ~7% increase in RMR reported with acute exposure to an even higher altitude of ~4300 m in women (Mawson et al., 2000), but smaller than the ~27% increase in RMR reported in men also with acute exposure altitude to ~4300 m (Butterfield et al., 1992). We also observed a return in RMR back to baseline values with more prolonged altitude exposure, with the ~5% increase in values at 36 hours being reduced to ~2% after 3 weeks of altitude exposure. A decrease in RMR back to sea level values has been observed at higher altitudes with RMR returning to baseline after 5 days of high-altitude exposure in women (Mawson et al., 2000), although in male subjects, RMR still remained elevated ~17% above sea level values with 3 weeks of high-altitude exposure (Butterfield et al., 1992). Notably, our study included a female-

only cohort and it is possible that sex-based differences exist for the effect of altitude on RMR. Hypoxia-inducible factor (HIF) is thought to play a role in the increased RMR seen at altitude (Palmer & Clegg, 2014), with evidence that estrogen may downregulate HIF activity in rodent models (Mukundan et al., 2004), providing some support for sex-based differences in RMR at altitude. Increased sympathetic activation is also thought to play a role in the increased RMR with altitude exposure (Moore et al., 1987), and there may be lower sympathetic support of RMR in women compared to men (Bell et al., 2001). Further studies are needed to investigate the presence of sex-based differences in RMR changes in response to mild and moderate altitude exposure in athletic cohorts. While reaching statistical significance, the magnitude of RMR change seen in our study must be considered. Indeed, the upper limit of the generally accepted 3-5% day-to-day variation in RMR (Compher et al., 2006) equates only to ~25-75 $kcal \cdot day^{-1}$; thus our findings are unlikely to have clinically significant implications for an athlete's total daily energy requirements.

While not reaching statistical significance (p=0.052), relative RMR at 36h-post was decreased by 1.6 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ compared to Pre-alt. This is similar to the 1.5 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ reduction measured following 12 days of altitude exposure in a case study of male (n=2) and female (n=2) rowers that was attributed to LEA during the altitude training camp (Woods, Garvican-Lewis, et al., 2017). The ~0.5 kg decrease in fat mass for athletes in the HEA group cannot explain this ~60 $kcal \cdot day^{-1}$ decrease in RMR from pre- to post-altitude, as this would result in an absolute reduction in RMR of ~2.3 $kcal \cdot day^{-1}$ (Wang et al., 2010). As such, it appears that the physiological adaptations that occurred with altitude training may be responsible for this 1.6 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$ reduction in RMR. An improved mitochondrial efficiency with altitude training (Gore et al., 2007; Murray & Horscroft, 2016) could contribute to a reduced RMR given that mitochondrial parameters have been linked to RMR in humans

(Larsen et al., 2011). Furthermore, in rodents, weight loss-induced decreases in RMR have been attributed to improved mitochondrial efficiency in skeletal muscle (Ferrara et al., 2023). Given this finding, it is possible that this previously reported reduction in RMR was due to adaptations that occurred with altitude exposure rather than LEA during the 12 days at altitude (Woods, Garvican-Lewis, et al., 2017). Alternatively, increases in training load during the altitude training camp may have altered RMR as this has been seen following periods of intensified training, although this may have been due to concurrent LEA as an increased training load may not have been matched with an increased EI (Woods et al., 2018). Future studies are needed to determine if there is a reduction in RMR upon return to sea level following altitude training camps independent of EA status and changes in training load, and if so, the duration of this suppression and the mechanism for this change.

6.5.2 RMR with LEA exposure

Despite a reduction in RMR independent of changes in body composition being an outcome within the REDs model (Mountjoy et al., 2023), we did not find any effect of 7 days of LEA on RMR while at altitude. Interestingly, the majority of athletes in the LEA group had an unchanged RMR following the 7-day period of LEA whereas among athletes in the HEA group, there was greater inter-individual variation when examining changes in RMR across this final week (see Figure 6.3). Notably, despite a RMR ratio commonly being used as an indicator of LEA (Sterringer & Larson-Meyer, 2022) most of the evidence supporting the use of a RMR as an indicator of LEA comes from cross-sectional studies demonstrating differences in RMR between athletes with and without indicators of LEA (Koehler et al., 2016; Lee et al., 2020; Melin et al., 2015; Myerson et al., 1991; Staal et al., 2018; Strock et al., 2020; Tornberg et al., 2017). Indeed, evidence of manipulations in LEA suppressing RMR in athletic populations is limited. A case report of a male combat athlete found a reduction in RMR (-149 kcal·day^{-1}) following 7 weeks of reduced EA (~20 kcal·kg FFM^{-1}·day) followed by 5 days of further

restrictions in EA (-4-9 kcal·kg FFM^{-1}·day^{-1}) (Langan-Evans et al., 2021), and a study of female athletes demonstrated a reduction in RMR (-65 kcal·day^{-1}) following 10 days of reduced EA (~25 kcal·kg FFM^{-1}·day^{-1}) (Oxfeldt et al., 2023). On the other hand, 3 days of a similarly reduced EA (~19 kcal·kg FFM^{-1}·day^{-1}) in male athletes did not result in changes to RMR (Kojima et al., 2020). When assessing why different outcomes of LEA exposure occur, both the characteristics of LEA exposure, and/or moderating factors must be considered (Burke et al., 2023). Altitude exposure may be a moderating factor that alters the physiological outcomes of LEA. For instance, reductions in sympathetic nervous system activity are thought to contribute to reductions in RMR with LEA (Müller & Bosy-Westphal, 2013). Yet, altitude exposure is thought to increase sympathetic nervous system activity (Sander, 2016). As such, it is possible that the altitude exposure altered the response to LEA exposure and a decrease in RMR would have been observed if the same LEA exposure occurred at sea level. Alternatively, a more prolonged and/or severe exposure of LEA may be needed to impact RMR.

6.5.3 Markers of LEA

The updated International Olympic Committee consensus statement on REDs provides new guidelines for diagnosing and assessing the risk of REDs using a mixture of primary and secondary LEA indicators, as well as emerging indicators that require more research before being fully endorsed as indicators of LEA (Stellingwerff et al., 2023). Among the LEA indicators that we assessed, T3 was the only one that was affected by the 7-day period of LEA, strengthening its use as a primary indicator of REDs (Stellingwerff et al., 2023). Interestingly, there was no association between change in T3 levels over the training camp, and change in RMR over the 7-day period of LEA. Other measured indicators showed inconsistent changes and seemed altered by altitude exposure and/or training rather than EA (see Figure 6.4 and 6.5). Despite other LEA indicators being present (Stellingwerff et al., 2023), no athlete presented with a RMR measurement considered suppressed across the training camp. Notably, a

suppressed RMR is listed only as an emerging indicator in the updated REDs CAT2 due to current concerns with specificity and sensitivity of measurement (Stellingwerff et al., 2023). Our results demonstrate that altitude exposure may be contributing to noise in this measurement and must be considered when measuring RMR in athletic cohorts. For instance, athletes undergoing RMR measurements at laboratories or institutions located at low to moderate altitude may present with an increased RMR if unacclimatised, leading to an artificially inflated RMR ratio or relative RMR. Additionally, measuring RMR in the periods following an altitude training camp should be used with caution until more research examining RMR following periods of altitude training is conducted.

6.5.4 Energy needs at altitude

The diet provided to athletes in the HEA group was aimed at providing optimal energy availability. Yet, meaningful reductions in fat mass occurred for some athletes in the HEA group (n=5), suggesting that study diets provided insufficient energy for these athletes. It is possible that athletes were not compliant with the dietary intervention despite the best efforts of the research team to ensure adherence, such as weighing and monitoring meals and taking into consideration individual food preferences. However, it is also possible that study diets did not provide sufficient energy. Of the five athletes in the HEA group that had a reduction in fat mass that exceeded the LSC, four maintained an elevated RMR during the final week. The remaining athlete was unique in also recording a reduction in FFM in addition to fat mass, potentially explaining the observed reduction in RMR. On the other hand, the remaining four athletes in the HEA group that maintained fat mass had a return in RMR back to Pre-alt levels at Wk3-alt. This, along with the negative correlation between changes in fat mass over the training camp and changes in RMR over the final 7 days at altitude ($r=-0.735$; $p=0.024$), suggests that athletes in the HEA group who maintained an increased RMR with altitude exposure were more likely to experience reductions in fat mass. This loss in fat mass may be

due to an underestimation of their energy requirements due to increases in RMR with altitude exposure altering the EA (40-45 kcal·kg FFM^{-1}·day^{-1}) that is recommended to support all physiological systems at sea-level (Melin et al., 2019). However, even if an "optimal" EA threshold could be determined for each athlete within this cohort at altitude, there are known complexities and nuances with the EA equation (Burke et al., 2018). Additionally, the estimation of EEE from training at altitude was determined from metabolic testing data conducted at sea-level, with the possibility of EEE being increased at altitude due to changes in metabolic pathways (Young et al., 2019). Finally, it is possible that physiological adaptations at altitude increase energy needs via mechanisms outside of RMR that were not accounted for in the EA equation, such as an increased excess post-exercise oxygen consumption (Navalta et al., 2018). Early studies at high altitudes in women reported an increase in total energy requirements beyond what could be accounted for by changes in RMR or EEE, which was termed "energy requirement excess" (Mawson et al., 2000). Given this, further research is needed to assess if physiological adaptations with altitude alter another component that contributes to daily energy needs.

6.6 Conclusion

In conclusion, RMR was transiently increased in female endurance athletes while living and training at altitude but was unaffected by LEA exposure. The increase in RMR observed was small (50-75 kcal·day^{-1}) and is unlikely to have clinically significant implications for an athlete's total daily energy requirements. However, RMR represents only one component of daily energy requirements, and physiological adaptations that occur with altitude may alter other components that contribute to daily energy needs. Given the downward trend in RMR that was seen upon return to sea-level, care should be taken when measuring and interpreting the RMR of athletes immediately post-altitude. Future studies are needed to determine if other components of total daily energy expenditure are altered with altitude exposure, what the

impact of EA status on these alterations may be, and if there are further sex-based differences in RMR changes in response to altitude exposure in athletic cohorts.

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Conflict of Interest

The authors declare no conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and fabrication, falsification, or inappropriate data manipulation.

6.7 Interlinking chapter

The second study of this thesis demonstrated a temporal effect of low altitude exposure on RMR such that RMR was increased with acute exposure and two weeks altitude exposure but was no longer elevated at three weeks altitude exposure. RMR at altitude was not impacted by 7 days of LEA. An unexpected downward trend in RMR post-altitude was also observed. This was unlikely to be due to LEA but may have rather been due to physiological adaptations with altitude exposure and/or increases in training load.

The third study of this thesis examined the need to standardise diet and exercise prior to a RMR measurement. This was achieved by manipulating EI and EEE for the 24 hours prior to a RMR measurement.

7 Study 3 Impact of Acute Dietary and Exercise Manipulation on RMR Measurements and DXA Body Composition Estimates

Publication statement:

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The chapter does not differ from the published paper apart from tables, figures and references, which have been renumbered to maintain consistency within the thesis.

7.1 Abstract

Purpose: To investigate the effects of acute diet and exercise manipulation on resting metabolic rate (RMR) measurement variability and dual energy x-ray absorptiometry (DXA) body composition estimates.

Methods: 10 male and 10 female endurance athletes (12 cyclists, 5 triathletes, 4 runners) of Tier 2 (n=18) to Tier 3 (n=2) calibre underwent five conditions using a Latin square counterbalance design. For 24 hours, athletes consumed a diet providing excessive energy availability (75 kcal·kg fat free mass $(FFM)^{-1}$) without exercise (GEA_{rest}), high energy availability (45 kcal·kg FFM^{-1}) without (HEA_{rest}) or with exercise (HEA_{ex}), or low-energy availability (15 kcal·kg FFM^{-1}) without (LEA_{rest}) or with exercise (LEA_{ex}). Exercise involved two bouts of cycling (morning bout: 149±34 min at 55% of maximal aerobic capacity (VO_2 max); afternoon bout: 60 min at 65% of VO_2 max) that resulted in a cumulative exercise energy expenditure of 30 kcal·kg FFM^{-1}. The following day, RMR and DXA measurements occurred after a 10 hour fast and 12 hours post-exercise.

Results: There were no sex differences in relative RMR (p=0.158) nor effects of any of the five conditions on RMR (p=0.358). For both male and female athletes, FFM estimates were decreased following the LEA_{rest} (-0.84±0.66kg; p=0.001) and LEA_{ex} (-0.65±0.86kg; p=0.016) conditions compared to the GEA_{rest} condition and following the LEA_{rest} (-0.73±0.51 kg; p=0.001) and LEA_{ex} (-0.54±0.79 kg; p=0.024) conditions compared to the HEA_{ex} condition. There was no effect of condition on fat mass estimates (p=0.819).

Conclusion: Acute periods of diet and exercise manipulation did not create artifacts in next-day RMR measurements. However, as changes in estimates of FFM were seen, diet and exercise should be controlled in the 24 hours prior to DXA scans.

7.2 Introduction

Adequate energy intake (EI) is needed to support optimal body function, making EI the foundation of the athlete's diet (Thomas et al., 2016). Insufficient EI may lead to low energy availability (LEA), where a mismatch exists between EI and exercise energy expenditure (EEE), leaving inadequate energy to support bodily function (Mountjoy et al., 2023). Problematic LEA is the underlying cause of Relative Energy Deficiency in Sport (REDs), a syndrome of impaired physiological function (Mountjoy et al., 2023). LEA may result in various health and performance consequences, making early identification of an athlete with LEA or REDs critical (Mountjoy et al., 2023).

An energy availability (EA) <30 kcal·kg FFM^{-1}·day^{-1} has previously been defined as "low" with EA calculated as follows: EI – EEE normalised to fat free mass (FFM) (Loucks et al., 2011). However, calculating an athlete's EA is not recommended for diagnostic purposes due to various difficulties and errors with this calculation; additionally, there is no universal threshold of EA that results in impaired function (Ackerman et al., 2023; Burke et al., 2018). Rather than attempting to directly calculate an athlete's EA, physiological outcomes of LEA are used to diagnosis REDs, and this often requires measuring multiple indicators of LEA (Ackerman et al., 2023; Stellingwerff et al., 2023).

A proposed physiological outcome of LEA within the REDs model is impaired energy metabolism resulting in a suppressed resting metabolic rate (RMR) (Mountjoy et al., 2023), with RMR representing the energy necessary to sustain normal bodily function at rest (Fullmer et al., 2015). A suppressed RMR has been suggested as an emerging indicator of REDs in athletes (Stellingwerff et al., 2023; Sterringer & Larson-Meyer, 2022). It is determined by the ratio of an athlete's laboratory-measured RMR via indirect calorimetry to their estimated RMR

using a prediction equation and/or measured RMR relative to FFM with values below a standardised threshold used to indicate a suppressed RMR (Loucks et al., 2011; Strock et al., 2020). However, because of RMR determination specificity and sensitivity concerns, the use of RMR measurements as diagnostic indicators of REDs are still emerging and not considered formal diagnostic criteria (Stellingwerff et al., 2023). Before RMR can be used as a diagnostic tool for REDs, factors that contribute to the 3-5% day-to-day variability in RMR measurements must be identified (Compher et al., 2006). From this, standardised procedures for measuring athletes' RMR can be developed to improve measurement precision.

Guidelines for determining RMR in healthy adults recommend that measurements occur in a >7 hour fasted state or after an overnight fast (Fullmer et al., 2015). Measuring RMR in a fasted state is implemented to avoid an artificially inflated RMR measurement from increased post-prandial metabolism known as the thermic effect of food (TEF). This is the energy expenditure of digesting, absorbing, and metabolizing dietary nutrients (Calcagno et al., 2019). However, this guideline assumes that TEF will no longer be present following an overnight fast, regardless of total EI the previous day. Because greater EI leads to a greater TEF (Quatela et al., 2016), it has been speculated that residual TEF could still be present after an overnight fast following a large EI, leading to a falsely elevated measured RMR (Diaz et al., 1992; Joosen et al., 2005). This might be especially relevant to athletes with large EEE leading to a large EI. In addition, prior exercise must be considered due to the increased metabolic rate post-exercise known as excess post-exercise oxygen consumption (EPOC) (Børsheim & Bahr, 2003). There is no clear guideline pertaining to the time participants should refrain from exercise prior to RMR measurements (Fullmer et al., 2015). Yet, like TEF, if RMR is measured while EPOC is present, then RMR measurements will also be falsely elevated.

In addition to considering the effects of total EI and EEE on TEF and/or EPOC, the resulting energy flux must be noted. A high energy expenditure with matched levels of EI results in high energy flux, whereas low energy expenditure with matched levels of EI results in low energy flux (Melby et al., 2019). In conditions of energy balance, an increased RMR has been reported in situations of high versus low energy flux (Bell et al., 2004). Although this could be due to an artificial inflation of RMR measurement via increased TEF or EPOC, it has been hypothesised that energy flux *per se* may be a determinant of RMR via changes in the sympathetic contribution to RMR (Bell et al., 2004). This represents another avenue by which prior EI and EEE may contribute to variability in RMR measurements. As such, the aim of this study was to investigate the effect of acute manipulation of EI and exercise on RMR measurements and body composition estimates via dual-energy X-ray absorptiometry (DXA). It was hypothesised that exercise would increase next day RMR measurements in both male and female athletes.

7.3 Methods

7.3.1 Participants

Ten male and ten female endurance-trained athletes of Tier 2 (n=18) to Tier 3 (n=2) calibre (McKay et al., 2022) were recruited for participation in this study (Table 7.1) with this sample size being calculated to detect differences in peak fat oxidation (PFO) relative to FFM between the sexes with 90% statistical power and an alpha of 0.05. Athletes included cyclists (n=11), triathletes (n=5) runners (n=3), and one athlete that trained for both cycling and running events (n=1). All female athletes were combined monophasic oral contraceptive pill (COC) users with testing occurring during the active pill phase of the COC cycle. All had used COC for >3 months prior to commencing the study with the mean length of usage being 7.1±8.5 years. COC brands included: Femme-Tab (n=2), Levlen ED (n=2), Micronelle 30 ED (n=1), Evelyn 150/30 ED (n=1), Yasmin (n=1), Isabelle (n=1), Estelle-35 ED (n=1), and Yang (n=1). Seven

participants habitually consumed their COC in the morning, and the remaining three in the evening. Participants that habitually consumed their COC in the morning were required to consume their COC after completing measurements on the day of testing. Details on COC preparations can be found as supplementary material on the journal website. The study was approved by the Human Ethics Research Committee at Australian Catholic University and informed consent was received from all athletes prior to participation.

Table 7.1. Athlete characteristics. Data presented as mean ± SD. BMI, body mass index; VO_2 max, maximal aerobic capacity; PPO, peak power output. *Significant using a Mann-Whitney-Wilcoxon Test.

	Male athletes	Female athletes	p-value
Age (yrs)	37.8±8.9	33.1±6.9	<0.001*
BMI ($kg \cdot m^2$)	25.2±3.4	23.7±3.3	0.012*
VO_2 max ($ml \cdot kg^{-1} \cdot min^{-1}$)	50.9±10.8	44.3±7.7	0.041*
PPO ($wattage \cdot kg^{-1}$)	4.4±1.0	4.1±0.6	0.379

7.3.2 Experimental protocol

7.3.2.1 Baseline testing

Prior to commencing the first experimental condition, athletes attended the laboratory for baseline testing. Baseline testing included a RMR measurement, DXA scan, and a maximal exercise test on a cycle ergometer. The information from these measurements was used to determine dietary and exercise targets for the 5 experimental conditions outlined below.

7.3.2.2 Experimental conditions overview

Using a Latin square counterbalance design, each participant underwent 5 conditions with an average washout of 8 days between conditions (Figure 7.1). Day 1 of each condition involved 24 hours of diet and exercise standardisation. Day 2 involved 24 hours of EI and EEE manipulation to achieve an EA target through diet alone or in combination with exercise. Day 3 featured the RMR measurement and a DXA scan followed by a blood draw.

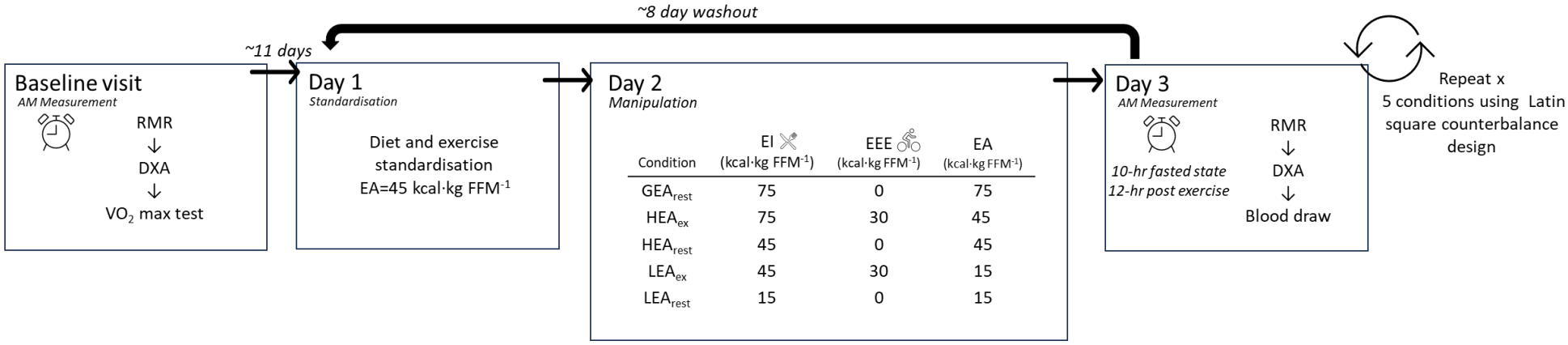

Figure 7.1. Study overview. Using a Latin square counterbalance design, athletes underwent five conditions of diet and exercise manipulation. Energy availability targets were achieved through diet alone or in combination with exercise. EI, energy intake; EEE, exercise energy expenditure; EA, energy availability; RMR, resting metabolic rate; DXA, dual-energy X-ray absorptiometry; VO_2 max test, maximal oxygen uptake test.

7.3.3 Dietary manipulation

All foods and beverages were weighed and provided to athletes for both day 1 (standardisation) and day 2 (manipulation) of each condition. Athletes self-reported any deviations from the study diet. Day 1 of dietary standardisation provided an EA of 45 kcal·kg FFM^{-1} with 55% energy from carbohydrates, 25% from protein and 20% from fat. Athletes self-selected if they engaged in exercise during this standardised period and reported this to the research team prior to the study so that EI could be set to maintain an EA of 45 kcal·kg FFM^{-1}. We allowed athletes in this study to self-select exercise on day 1 to minimise interference with an athlete's training given the extensive control of exercise that occurred on day 2 across 5 conditions. However, athletes replicated the exercise performed during the standardisation period for each of the 5 conditions. As such, an athlete's day 1 EI and EEE were the same for each of the 5 standardised periods.

Day 2 involved one of five conditions: EA that would result in body mass gain if consumed chronically without exercise (GEA_{rest}; 75 kcal·kg FFM^{-1}), high EA with exercise (HEA_{ex}; 45 kcal·kg FFM^{-1}), high EA without exercise (HEA_{rest}; 45 kcal·kg FFM^{-1}), LEA with exercise (LEA_{ex}; 15 kcal·kg FFM^{-1}), and LEA without exercise (LEA_{rest}; 15 kcal·kg FFM^{-1}). Using the FFM from the baseline scan and corresponding EA target, EI was calculated as followed:

$$EI = (\text{Target EA} \times FFM) + EEE$$

$$GEA_{rest} = 75 \text{ kcal·kg } FFM^{-1} \times FFM$$

$$HEA_{ex} = (45 \text{ kcal·kg } FFM^{-1} \times FFM) + 30 \text{ kcal·kg } FFM^{-1}$$

$$HEA_{rest} = 45 \text{ kcal·kg } FFM^{-1} \times FFM$$

$$LEA_{ex} = (15 \text{ kcal·kg } FFM^{-1} \times FFM) + 30 \text{ kcal·kg } FFM^{-1}$$

$$LEA_{rest} = 15 \text{ kcal·kg } FFM^{-1} \times FFM$$

This resulted in three different study diets, as diets were the same for the GEA_{rest} and HEA_{ex} condition (EI= 75 kcal·kg FFM^{-1} × FFM) and the HEA_{rest} and LEA_{ex} condition (EI=45 kcal·kg FFM^{-1} × FFM) but differed in levels of EEE. Conditions with the same EA but differing EEE (HEA_{ex} vs. HEA_{rest} and LEA_{ex} vs. LEA_{rest}) allowed for the effects of EPOC to be assessed; the conditions without exercise but varying EI (GEA_{rest}, HEA_{rest}, and LEA_{rest}) allowed the effect of the TEF to be assessed; and the two conditions of high EA with and without exercise (HEA_{ex} and HEA_{rest}) allowed the effect of energy flux to be assessed. The macronutrient distribution was the same for all conditions: 55% carbohydrate, 25% protein, and 20% fat. The TEF was calculated for each condition with the metabolic cost of fat being 2.5%, carbohydrate 7% and protein 27.5% (Jéquier, 2002). Athletes were required to space out meals and snacks by >1 hour, and to consume the last snack 10 hours prior to the RMR measurement so that RMR measurements always occurred in a 10-hour fasted state. Athletes who were habitual caffeine drinkers were permitted to consume caffeine but were required to replicate caffeine intake across all conditions. The requirement that athletes be 10 hours fasted on the morning of day 3 further ensured that guidelines for caffeine intake prior to RMR testing were followed given the recommendation to refrain from caffeine for >4 hours prior to RMR measurements (Compher et al., 2006).

7.3.4 Exercise manipulation

For the two conditions involving exercise (HEA_{ex} and LEA_{ex}), the EEE of 30 kcal·kg FFM^{-1} was achieved by two bouts of cycling on a stationary load bike (Load Excalibur Sport, Groningen, Netherlands) during day 2. This included a morning bout at a wattage corresponding to 55% of maximal aerobic capacity (VO_2max) as determined during baseline testing (means: males 157±40 W, females 103±16 W) that lasted on average 135±26 minutes for male athletes and 163±37 minutes for female athletes, and an afternoon bout of cycling at 65% of VO_2max (means: males 195±46 W, females 131±19 W) for 60 minutes. The afternoon

session was scheduled to end 12 hours prior to the RMR measurement. The duration of the morning session was determined as followed:

Duration= (30 kcal·kg FFM^{-1} – EEE_{kcal} of afternoon session)/$EEE_{kcal/min}$ at 55% of VO_2 max

The EEE at each intensity of cycling was prospectively determined from the gas exchange data collected during the maximal exercise test performed at the baseline visit (Weir, 1949) as such:

$$EEE\ (kcal/min) = [(3.94 \times VO_2) + (VCO_2 \times 1.11)] – (24\ h\ RMR/1440)$$

For the remaining three conditions (GEA_{rest}, HEA_{rest}, LEA_{rest}), athletes remained physically inactive for the 24 hours prior to RMR measurements. This involved no planned exercise beyond activities of daily living.

7.3.5 Measurements

7.3.5.1 Resting metabolic rate

RMR was measured upon arriving to the laboratory on the morning of day 3 with individual repeat measurements occurring within a 30 minute window to minimise effects of circadian changes on RMR (Zitting et al., 2018). Athletes were 10 hours fasted (including nil fluid intake) for this measurement and did not engage in any physical activity prior to this measurement beyond the exercise performed as part of the condition the day prior (12 hours post-exercise). Athletes first rested in a dark and quiet room for 10 minutes before being given a one-way mouthpiece for a further 15-minute familiarisation period. This protocol of rest and familiarisation produces comparable results to RMR measured in an inpatient setting (Bone & Burke, 2018). The familiarisation period was followed by two consecutive data collection periods during which expired air was collected into Douglas bags with a 10-minute data collection per bag. Bags were analysed using ametek Oxygen (O_2) and carbon dioxide (CO_2) analysers (VacuMed, Ventura, CA) that were calibrated with known concentrations of O_2 and

CO_2 before use. The expirate from each bag was sampled for 1 minute and the gas sampling time and flow rate were recorded. The volume of the remaining expirate was then determined using a Tissot spirometer via an evacuation pump. The average of the two bags was used to calculate absolute RMR over 24 hours ($kcal \cdot day^{-1}$). The absolute RMR was used to calculate relative RMR ($kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$) and a RMR ratio (measured:predicted) using four RMR predictive equations. Predictive equations included the Harris benedict equation (Harris & Benedict, 1918), $Cunningham_{1980}$ equation (Cunningham, 1980), $Cunningham_{1991}$ equation (Cunningham, 1991), and a calculation from the sum of tissue specific metabolic rates of DXA-estimated brain, skeletal muscle, adipose tissue, bone and residual mass (Hayes et al., 2002). An athlete was classified as having a suppressed RMR when the calculated RMR ratio (Strock et al., 2020) and/or relative RMR (Loucks et al., 2011) fell below a standardised threshold at outlined in Table 7.2.

Table 7.2. Thresholds used to indicate a suppressed resting metabolic rate (RMR) with each predictive equation and severe primary, primary, secondary, and emerging low energy availability indicators as per the updated REDs CAT2. RMR, resting metabolic rate; HB, Harris Benedict; DXA, dual-energy X-ray absorptiometry; T3, triiodothyronine; TC, total cholesterol; LDL, low density lipoprotein; IGF-1, insulin-like growth factor 1.

Suppressed RMR	RMR_{ratio} <0.90 using HB equation (Harris & Benedict, 1918)
	$RMR_{ratio:}$ <0.90 using $Cunningham_{1980}$ equation (Cunningham, 1980)
	RMR_{ratio} <0.92 using $Cunningham_{1991}$ equation (Cunningham, 1991)
	RMR_{ratio} <0.94 using DXA prediction (Hayes et al., 2002)
	Relative RMR <30 kcal·kg FFM^{-1}·day^{-1}
Severe primary LEA indicator	Total testosterone <8.3 nmol/L (males only), Free testosterone <255 pmol/L (males only), T3 <3.5 pmol/L
Primary LEA indicator	Total testosterone: 8.3-13.5 nmol/L (males only), Free testosterone: 255-373 pmol/L (males only), T3 3.5-4.1 pmol/L
Secondary LEA indicator	TC >5.2 mmol/L, LDL >3.4 mmol/L
Emerging LEA indicator	Cortisol >620 nmol/L, IGF-1 below lowest quartile of age dependent range

7.3.5.2 Indicators of LEA

With each visit, indicators of LEA were measured, and cut-offs implemented as per the Relative Energy Deficiency in Sport Clinical Assessment Tool Version 2 (REDs CAT2) (Stellingwerff et al., 2023) as outlined in Table 7.2. This included primary indicators (outcome parameters with strong evidence that they result from problematic LEA), secondary indicators (outcome parameters with some scientific evidence that they result from problematic LEA), and potential indicators (emerging outcome parameters lacking robust scientific evidence but linked to problematic LEA) (Stellingwerff et al., 2023). All indicators of LEA were measured in both male and female athletes, with the exception of testosterone, which is currently only an established indicator of LEA in male athletes (Stellingwerff et al., 2023). Insulin levels were also measured to assess changes in fasting concentrations across conditions but were not used as a potential indicator of LEA due to lack of a lower end lab-specific range.

7.3.5.3 Body composition

Immediately following the RMR measurement, participants had a scale body mass measured and then body composition was determined via DXA scan, using the Best Practice Protocols of the Australian Institute of Sport (Slater, Townsend, et al., 2023). This involved being overnight fasted (with nil fluid intake), voiding bladder before the scan, and using standardised positioning of athletes on the DXA scanning bed by using Velcro straps and positioning aids (Slater, Townsend, et al., 2023). Hydration status was assessed by a waking mid-stream urine sample for analysis of urinary specific gravity (USG). Athlete scans were performed in the same mode (GE Lunar iDXA) and analysed using GE encore by the same trained researcher to assess FFM, lean body mass (LBM), and fat mass (FM). Scans were analysed automatically by the DXA software, but all regions of interest were reconfirmed prior to analyses. DXA analysis was used to model brain mass, skeletal mass, bone mass, and adipose tissue mass that were then used to estimate RMR using tissue-specific metabolic rates (Hayes et al., 2002).

7.3.5.2 Blood samples

Blood samples were taken to assess indicators of LEA as per the REDs CAT2 (Stellingwerff et al., 2023) (see Table 7.2), which included the following: triiodothyronine (T3), cortisol, insulin-like growth factor 1 (IGF-1), insulin, total cholesterol, low density lipoprotein (LDL), total testosterone (males only), and free testosterone (males only). After the DXA scan and RMR measurements, a 17 mL venous blood sample was collected from an antecubital vein into a serum separator tube, with blood sample analysis performed using a chemiluminescent immunoassay through a commercial laboratory (Laverty Pathology, Bruce, ACT, Australia).

7.3.6 Statistical analysis

Statistical analyses were performed in R Studio (v3.5.2) using general linear mixed models with statistical significance accepted at an α level of $p \leq 0.05$. Fixed effects for the model included condition and sex, with subject ID as a random effect within the models. The initial model included all possible interactions, with non-significant interactions then dropped. BMI and subject ID were included as random effects for the models assessing relative RMR and RMR ratio with each predictive equation. Statistical significance of the fixed effect was determined using a Type II Wald tests with Kenward-Roger degrees of freedom. Where significant fixed effects were evident, a Tukey's post-hoc comparison was used to identify where differences exist. Non-normally distributed data (relative RMR, body mass, FFM, FM, free testosterone, T3, IGF-1, insulin, and LDL) were log-transformed for statistical analyses. Due to technical issues with the DXA machine, one female athlete could not undergo a DXA scan for the HEA_{ex} condition. The average of her DXA estimates from the remaining conditions was used for data analysis of relative RMR and RMR ratios in the general linear mixed model. However, her data was excluded from body composition analyses and when analysing the RMR ratio involving DXA estimates of tissue-organ components. Due to technical issues obtaining the blood sample from one female for the LEA_{ex} condition, her data was excluded for blood

marker analysis. Finally, one female athlete was missing T3 levels from the LEA_{rest} condition and a male athlete IGF-1 levels from the HEA_{ex} condition. For these participants, the average of remaining conditions was used for data analysis in the general linear mixed model.

7.4 Results

7.4.1 Dietary analysis

As intended, the GEA_{rest} and HEA_{ex} condition and the HEA_{rest} and LEA_{ex} condition had the same EI, macronutrient intake, and TEF ($p=1.000$), but differences were seen between all other conditions ($p<0.001$; Table 7.3). Likewise, the LEA_{ex} and HEA_{ex} condition had the same EEE ($p=1.000$). The EEE of male athletes was greater than female athletes for conditions involving exercise ($p<0.001$), and male athletes also had greater EI and TEF than female athletes for the GEA_{rest} and HEA_{ex} conditions ($p<0.05$). EA was slightly higher for female athletes compared to male athletes for the GEA_{rest} condition (~1 kcal· kg FFM^{-1}; $p=0.023$).

Table 7.3. Energy, carbohydrate, protein and fat intake, exercise energy expenditure, and thermic effect of food for male and female athletes during each condition. Data presented as mean ± SD. EA, energy availability; EI, energy intake; CHO, carbohydrate; EEE, exercise energy expenditure; TEF, thermic effect of food. [§]Significant vs. GEA_{rest}, HEA_{rest}, and $LEA_{rest,}$ [&]Significant vs. HEA_{rest}, LEA_{ex} and LEA_{rest}, *Significant vs. all other conditions, [$]Significant vs. GEA_{rest}, HEA_{ex} and $HEA_{rest.}$

	Male athletes					Female athletes				
	GEA_{rest}	HEA_{ex}	HEA_{rest}	LEA_{ex}	LEA_{rest}	GEA_{rest}	HEA_{ex}	HEA_{rest}	LEA_{ex}	LEA_{rest}
EA (kcal· kg FFM^{-1})	74±1.1*	45±0.9	45±0.8	15±0.4[$]	15±0.2[$]	75±0.7*	45±0.6	45±0.9	15±0.4[$]	15±0.3[$]
EEE (kcal)	-	1867±282[§]	-	1867±282[§]	-	-	1430±184[§]	-	1438±184[§]	-
EI (kcal)	4679±733[&]	4678±733[&]	2799±436	2799±436	934±139*	3576±465[&]	3578±466[&]	2145±269	2145±269	712±93*
CHO ($g \cdot kg^{-1}$)	7.9±0.8[&]	7.9±0.8[&]	4.8±0.5	4.8±0.5	1.6±0.2[*]	7.5±0.6[&]	7.5±0.6[&]	4.5±0.4	4.5±0.4	1.5±0.1[*]
Protein ($g \cdot kg^{-1}$)	3.6±0.4[&]	3.6±0.4[&]	2.2±0.2	2.2±0.2	0.7±0.1[*]	3.4±0.2[&]	3.4±0.2[&]	2.1±0.1	2.1±0.1	0.7±0.1[*]
Fat ($g \cdot kg^{-1}$)	1.3±0.1[&]	1.3±0.1[&]	0.8±0.1	0.8±0.1	0.3±0.02[*]	1.2±0.1[&]	1.2±0.1[&]	0.7±0.1	0.7±0.1	0.2±0.01[*]
TEF (kcal)	525±82[&]	525±83[&]	315±50	315±49	106±16*	401±52[&]	401±52[&]	240±31	240±31	82±11*

7.4.2 Resting metabolic rate

The absolute RMR of male athletes was greater than female athletes (p=0.0001), but when absolute RMR was expressed relative to FFM, there was no longer sex differences (p=0.158) (Table 7.4). There was no effect of condition on absolute RMR (p=0.665) or relative RMR (p=0.358). The RMR ratio calculated with each predictive equation did not differ between male and female athletes, nor with condition (p>0.05). The USG of male athletes was greater than female athletes (p=0.006), and the USG for the HEA_{ex} condition was greater than the LEA_{rest} condition (p=0.042).

Table 7.4. Absolute RMR, relative RMR, RMR ratio, and USG for each condition in male and female athletes. RMR, resting metabolic rate; HB, Harris Benedict; C1980, Cunningham 1980; C1991, Cunningham 1991; DXA, dual energy X-ray absorptiometry; USG, urine specific gravity. Data presented as mean ± SD. *Significant LEA_{rest} vs. $HEA_{ex.}$

	Male athletes					Female athletes					P		
	GEA_{rest}	HEA_{ex}	HEA_{rest}	LEA_{ex}	LEA_{rest}	GEA_{rest}	HEA_{ex}	HEA_{rest}	LEA_{ex}	LEA_{rest}	Sex	Condition	Inter.
RMR (kcal)	1894±208	1890±226	1844±292	1871±259	1838±197	1455±218	1503±247	1501±193	1565±245	1527±237	0.0001	0.665	0.253
RMR (kcal·FFM^{-1})	30.2±2.4	30.2±2.2	29.6±3.4	30.2±2.7	29.8±3.1	30.7±4.3	31.5±4.5	31.6±3.6	33.2±4.2	32.6±4.3	0.158	0.358	0.327
HB ratio	1.03±0.06	1.03±0.07	1.01±0.11	1.02±0.08	1.01±0.09	1.01±0.11	1.04±0.12	1.04±0.09	1.09±0.10	1.07±0.13	0.436	0.491	0.290
C1980 ratio	1.04±0.07	1.04±0.07	1.02±0.12	1.04±0.09	1.03±0.09	0.97±0.12	1.00±0.13	1.00±0.10	1.05±0.13	1.03±0.13	0.566	0.447	0.314
C1991 ratio	1.09±0.07	1.10±0.07	1.07±0.12	1.09±0.09	1.08±0.10	1.04±0.13	1.07±0.15	1.07±0.11	1.12±0.14	1.10±0.14	0.900	0.456	0.331
DXA ratio	1.08±0.07	1.07±0.06	1.06±0.12	1.08±0.09	1.07±0.09	1.04±0.15	1.09±0.16	1.08±0.12	1.13±0.17	1.12±0.15	0.708	0.328	0.307
USG	1.023±0.004	1.025±0.005	1.019±0.004	1.025±0.005	1.018±0.005*	1.017±0.005	1.020±0.003	1.017±0.006	1.017±0.009	1.018±0.006*	0.006	0.010	0.066

7.4.3 Body composition

Male athletes had a greater body mass (+15.8 kg; p=0.004), FFM (+15.6 kg; p<0.0001), and LBM (+14.9 kg; p<0.0001) than female athletes. There were no sex differences in FM (p=0.913), nor was there an effect of condition on FM estimates (p=0.819) (Figure 7.2). Body mass decreased following the LEA_{rest} (-0.82±0.81 kg; p=0.004) and LEA_{ex} (-0.56±0.93 kg; p=0.031) conditions compared to the GEA_{rest} condition. Differences were also seen for FFM estimates such that estimates were decreased following the LEA_{rest} (-0.84±0.66 kg; p=0.001) and LEA_{ex} (-0.65±0.86 kg; p=0.016) conditions compared to the GEA_{rest} condition and following the LEA_{rest} (-0.73±0.51 kg; p=0.001) and LEA_{ex} (-0.54±0.79 kg; p=0.024) conditions compared to the HEA_{ex} condition. Similarly, LBM was decreased following the LEA_{rest} (-0.85±0.65kg; p<0.001) and LEA_{ex} (-0.61±0.77kg; p=0.016) conditions compared to the GEA_{rest} condition and following the LEA_{rest} (-0.73±0.52 kg; p=0.001) and LEA_{ex} (-0.54±0.80 kg; p=0.027) conditions compared to the HEA_{ex} condition. Estimates of trunk FFM and LBM were decreased following the LEA_{rest} condition compared to the GEA_{rest} (FFM: -0.63±0.49 kg, LBM: -0.64±0.50 kg; p=0.0001), HEA_{ex} (FFM: -0.66±0.36 kg, LBM: -0.67±0.37 kg; p<0.0001) and HEA_{rest} (FFM: -0.39±0.56 kg; LBM: -0.37±0.55 kg; p<0.03) conditions and for the LEA_{ex} condition compared to the HEA_{ex} condition (FFM: -0.36±0.60 kg, LBM: -0.36±0.60 kg; p<0.05). Estimates of arm FFM (p=0.177) and LBM (p=0.201) were not different between conditions, and while there was a main effect of conditions for estimates of leg FFM (p=0.042) and LBM (p=0.044), post-hoc analysis was non-significant for both when comparing differences between conditions in male and female athletes (p>0.05). Regional estimates of arm (p=0.852), leg (p=0.907), and trunk (p=0.914) FM were also not different between conditions.

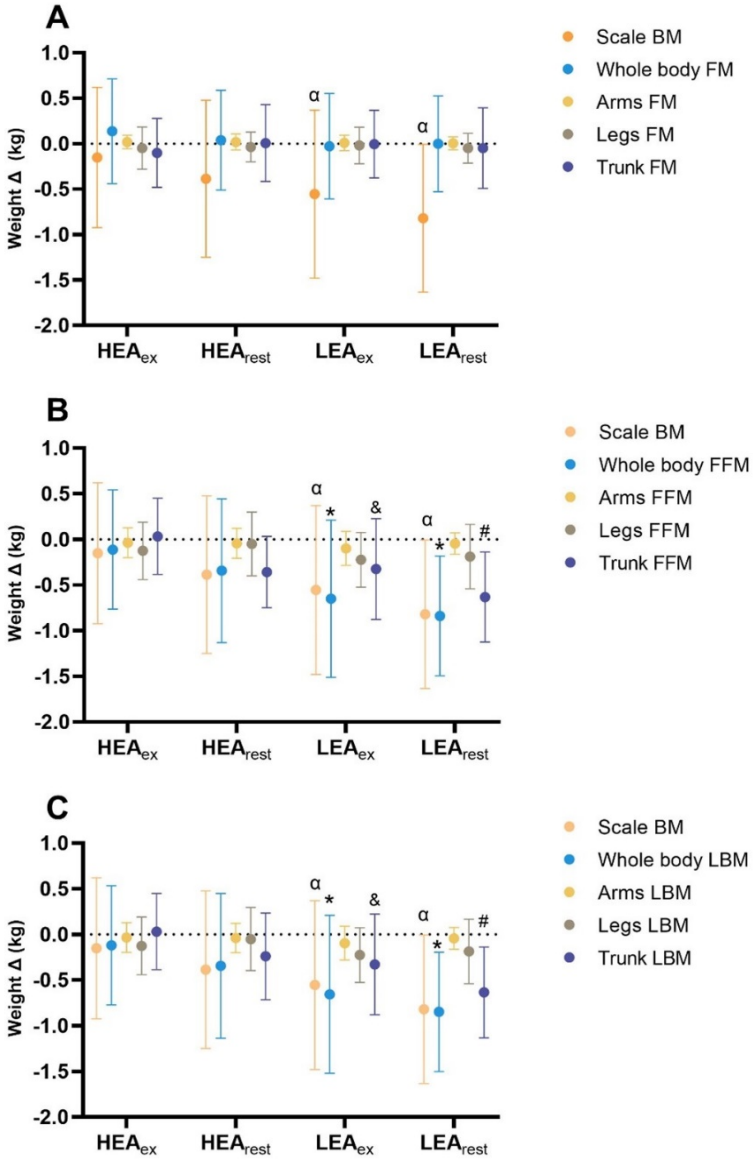

Figure 7.2. Difference in scale body mass and dual-energy X-ray absorptiometry body composition estimates in fat mass (A), fat free mass (B) and lean body mass (C) normalised to the GEA_{SED} condition in male and female athletes (n=10 M, 9 F). Data presented as mean ± SD. BM, body mass; FM, fat mass; FFM, fat free mass; LBM, lean body mass. $^{\alpha}$significant vs. $GEA_{rest.}$ *significant vs. GEA_{rest} and HEA_{ex}, $^{\#}$Significant vs. GEA_{rest}, HEA_{ex}, and HEA_{rest}, $^{\&}$Significant vs HEA_{ex}.

7.4.4 Indicators of LEA

Male athletes had a greater concentration of T3 ($p<0.0001$) and a lower concentration of cortisol ($p<0.0001$) and total cholesterol ($p=0.041$) than female athletes (Table 7.5). There was

an interaction effect for LDL ($p=0.029$), but post-hoc analysis was non-significant when comparing differences between conditions in male and female athletes. For both male and female athletes, cortisol concentrations were higher following the LEA_{rest} condition compared to the LEA_{ex} ($p=0.004$) and insulin was higher following the GEA_{rest} condition compared to all other conditions ($p<0.001$). While there was a main effect of conditions for IGF-1 concentrations ($p=0.02$), post-hoc testing revealed non-significant differences between conditions ($p>0.05$). There was no main effect of conditions on T3 ($p=0.115$) in both male and female athletes or total testosterone ($p=0.388$) or free testosterone ($p=0.301$) in male athletes.

Table 7.5. Indicators of low energy availability in each condition in male and female athletes (n=10M, 9F) as per the updated REDs CAT2 with corresponding reference range. Data presented as mean ± SD. T3, triiodothyronine; IGF-1, insulin-like growth factor 1; TC, total cholesterol; LDL, low density lipoprotein; T, testosterone. *Significant vs LEA_{ex}, &Significant vs all other conditions, #Significant vs GEA_{rest}.

	Male athletes					Female athletes					P		
	GEA_{rest}	HEA_{ex}	HEA_{rest}	LEA_{ex}	LEA_{rest}	GEA_{rest}	HEA_{ex}	HEA_{rest}	LEA_{ex}	LEA_{rest}	Sex	Condition	Inter.
T3 (pmol/L) 3.5-6.0 pmol/L	6.0±0.3	6.0±0.3	6.0±0.3	5.8±0.3	5.8±0.4	5.2±0.4	5.2±0.4	5.0±0.4	5.1±0.4	5.1±0.3	<0.0001	0.115	0.624
Cortisol (nmol/L) <620 nmol/L	425.8±90.3	412.1±122.6	459.3±135.0	366.4±101.4	493.0±124.7*	657.4±126.6	600.1±131.9	656.4±119.5	592.4±142.5	677.2±147.7*	<0.0001	0.002	0.883
IGF-1 (nmol/L)[a]	26.9±5.2	26.4±6.1	27.8±6.0	26.5±7.4	26.5±6.7	26.6±7.2	25.0±6.6	27.1±6.7	25.1±9.2	24.8±6.8	0.631	0.020	0.874
Insulin (mU/L) <10 mU/L	6.7±2.7&	4.6±2.4	4.9±3.5	4.4±2.5	3.8±1.8	8.6±5.1&	6.0±3.8	4.9±2.8	5.0±2.9	4.9±3.0	0.536	<0.0001	0.869
TC (mmol/L) <5.2 mmol/L	4.3±0.8	4.4±1.1	4.5±1.0	4.4±1.0	4.5±0.9	5.1±0.5	4.8±0.6	5.3±0.5	5.2±0.7	5.3±0.7	0.041	0.112	0.302
LDL (mmol/L) <3.4 mmol/L	2.2±0.6	2.6±0.9	2.4±0.8	2.6±0.9	2.6±0.9#	2.8±0.7	2.6±0.5	3.0±0.4	2.9±0.6	3.0±0.8	0.160	0.017	0.029
Total T (nmol/L) 8.3-29 nmol/L	17.0±3.8	16.0±2.9	15.8±2.6	15.9±2.4	17.2±5.1	-	-	-	-	-	-	0.388	-
Free T (pmol/L) 255-720 pmol/L	370.8±76.4	356.3±81.8	338.3±90.8	344.9±93.6	362.4±94.4	-	-	-	-	-	-	0.301	-

[a]Reference range varied with sex and age of participant

All athletes presented with at least one primary, secondary, or potential indicator of LEA during the course of the study (see supplementary data on journal website). The most common indicator of LEA was low free testosterone in male athletes (n=25 incidences) and elevated cortisol in female athletes (n=33 incidences) whereas the least common indicator was low T3 (n=0 incidences). A suppressed RMR was seen for at least one RMR measurement for 16 athletes (n=8 M, 8 F) and this was mostly due to relative RMR falling below the standardised threshold (n=38 incidences).

7.5 Discussion

The major findings of this study are that neither an acute period of excessive or inadequate EI nor large workloads of moderate intensity, continuous exercise altered measurements of next day RMR. This suggests that TEF and EPOC did not carry over into the next day and did not lead to artificial increases in RMR measurement, nor did energy flux alter RMR measurements. However, unlike RMR measurements, body mass and DXA body composition estimates of LBM and FFM were influenced by prior EI and EEE. As DXA scans are often performed alongside RMR measurements to assist in the interpretation of findings, this may indirectly impact guidelines for RMR measurements. Although artifacts in body composition estimates likely represent a small error, this may be significant when an athlete's relative RMR or RMR ratio is close to the threshold indicative of a suppressed RMR.

While it is generally accepted that RMR measurements should occur in a fasted state due to the increased metabolic rate that occurs post-prandially (Fullmer et al., 2015), EI during the day prior to testing is rarely considered. For instance, a review of studies that have used a RMR ratio as an indicator of LEA found that 10 of the 13 studies measured RMR in an overnight fasted state or >7 hour fasted state, with the remaining three studies not providing information on the fasting status of participants (Sterringer & Larson-Meyer, 2022). Yet, no study provided

information on standardising diet the day prior to testing despite EI being one of the factors that influences the time needed for metabolic rate to return to baseline values post-prandially (Quatela et al., 2016). In support of prior EI contributing to variability in RMR measurements, a residual TEF was speculated to cause an increased RMR measured in an overnight fasted state that could not be explained by changes in body composition following 14 days (Joosen et al., 2005) and 42 days (Diaz et al., 1992) of overfeeding. Yet, the energy content of these "overfeeding" diets (3500-4700 kcal·day^{-1}) (Diaz et al., 1992; Joosen et al., 2005) believed to create artifacts in RMR measurements from residual TEF may be similar to athletes' energy requirements during phases of high training loads (Heydenreich et al., 2017). As such, we were interested in investigating if TEF could contribute to RMR measurement variability in athletes. Among the dietary conditions implemented in this study, TEF ranged from ~105 kcal for male athletes and ~80 kcal for female athletes when consuming the LEA_{rest} diet versus ~525 kcal for male athletes and ~400 kcal for female athletes when consuming the GEA_{rest} or HEA_{ex} diet (Table 7.3). Despite these apparent differences in TEF between conditions, there was no effect on RMR, suggesting no residual TEF when RMR was measured in a 10-hour fasted state. To our knowledge, no study has assessed changes in RMR measured in an overnight fasted state following acute periods of energy manipulations, but an increased sleeping metabolic rate (SMR) measured with whole body indirect calorimetry has been reported following 24 hours of overfeeding (Thearle et al., 2013; Vinales et al., 2017). As SMR was determined by the mean metabolic rate from 11:30 pm to 5:00 am, with measurements commencing 4.5 hours post-prandial, it's possible that the increased SMR with acute overfeeding was due to TEF persisting throughout a portion of the testing window (Thearle et al., 2013; Vinales et al., 2017). However, the time course of change in metabolic rate throughout the night was not reported, so the time for metabolic rate to return to baseline following acute periods of overfeeding remains unknown. Overall, the results of our study suggest that EI the day prior to RMR measurements

does not need to be standardised. Rather, athletes should continue to match EI with nutrition goals during this time, and RMR measurements should occur in a ≥10 hour overnight fasted state so that measurements are devoid of TEF.

Following the cessation of exercise, metabolic rate remains elevated– greatest immediately post-exercise and then progressively declining due to EPOC (Børsheim & Bahr, 2003). If RMR is measured while EPOC is still present, then RMR measurements will be falsely elevated. However, it is unclear how long participants need to refrain from exercise prior to RMR measurements (Fullmer et al., 2015). Of the 13 studies in the previously mentioned review that have used a RMR ratio as an indicator of LEA, seven reported restricting exercise for >24 hours prior to the RMR measurement (Sterringer & Larson-Meyer, 2022). Yet, abstaining from exercise the day prior to testing to ensure that RMR measurements are devoid of EPOC may not be a realistic guideline for many high-level athletes. The time required for metabolic rate to return to baseline following exercise depends on various factors, including both exercise intensity and duration (Laforgia et al., 2006; Panissa et al., 2021). We implemented an exercise protocol that mirrors the real-life training plans of endurance athletes with both a morning and evening session of differing intensities (55% and 65% VO_2). This resulted in an average cumulative cycling duration of ~210 minutes. In contrast to our findings that suggests EPOC was no longer present 12 hours post-exercise, RMR has reported to be elevated by ~4% in sedentary women 22 hours post cycling at 50% VO_2 max for 60 minutes (Hunter et al., 2017) and by ~5% in trained males 18 hours post running at 50% VO_2 max for 3 hours (Bielinski et al., 1985). These contrasting results may be due to differences in the training status of participants, with untrained individuals having a slower return of EPOC post-exercise (Børsheim & Bahr, 2003). Alternatively, the mode of exercise may create differences, as running may result in a greater EPOC compared to cycling (Cunha et al., 2016). In support of

our findings, RMR measurements have shown to be unchanged 15 hours post running at 60% of VO_2max for a duration that resulted in an EEE of 500 kcal in men and women (Kang et al., 2020) and 14.5 hours post 60 minutes cycling at 50% VO_2 max in physically active males (Gillette et al., 1994). As the magnitude and duration of EPOC will differ with high intensity exercise and resistance exercise (Børsheim & Bahr, 2003), further research is needed to assess if this type of exercise needs to be restricted in the 24 hours prior to RMR measurements, as well as other aspects of sport that may contribute to variability in RMR measurements. For instance, fluctuations in RMR have been reported across match week in male soccer players (Carter et al., 2023) and rugby league players with an increased RMR following game day thought to be from muscle damage associated with collisions (Hudson et al., 2020). Our results suggest that athletes can engage in endurance exercise to which they are accustomed at a low to moderate intensity without impacting next day RMR measurements.

Unlike EPOC and TEF, which can create an artifact and erroneous RMR measurements, energy flux is hypothesised to be a determinant of RMR via influencing sympathetic support of RMR (Bell et al., 2004). Consistent with this, RMR was 3% above baseline following 10 days of increasing both EEE and EI by 1100-1300 kcal·day^{-1}, creating a state of high energy flux in men of unknown athletic calibre (Goran et al., 1994). Shorter periods of energy flux manipulation have also influenced RMR, with a greater RMR seen after three days of high energy flux (EI: ~4400 kcal·day^{-1}, EEE~1500 kcal·day^{-1}) compared to three days of low energy flux (EI: ~2600 kcal·day^{-1}, EEE: ~ kcal·day^{-1}) in trained men (Bullough et al., 1995). A state of increased energy flux has been proposed to help with weight loss maintenance, with a greater RMR apparent after four days of high energy flux (EI: ~3200 kcal·day^{-1}, EEE: ~500 kcal·day^{-1}) compared to low energy flux (EI: ~2500 kcal·day^{-1}, EEE: ~0 kcal·day^{-1}) that was preceded by a 7% loss of body mass over an 8-12 week period in males and females with obesity (Paris

et al., 2016). It is possible that these previous reports of increased RMR under high energy flux versus low energy flux conditions were an artifact of residual EPOC and TEF, as increased EEE and EI is required to achieve a state of high energy flux. However, our results suggest the TEF and EPOC do not carry over to next day RMR measurements. In contrast to studies showing an effect of energy flux on RMR, we found an absence of RMR differences between conditions of high energy flux (HEA_{ex}: EI: ~4128 kcal·day^{-1}, EEE ~1648 kcal·day^{-1}) and low energy flux (HEA_{rest}: EI: ~2472 kcal·day^{-1}, EEE ~0 kcal·day^{-1}). This may be due to differences in the periods of energy flux, as 24 hours of dietary and exercise manipulation may have been insufficient to create changes in the sympathetic support of RMR.

Using standardised thresholds (Strock et al., 2020), 16 of the 20 athletes presented with at least one RMR measurement that was considered suppressed, with the majority of suppressed RMR measurements due to a relative RMR <30 kcal·kg FFM^{-1}·day^{-1}. For athletes with greater FFM, this may be a false negative as the RMR to FFM ratio is reduced with increased FFM due to an increased proportion of FFM as low metabolic rate tissue (Heymsfield et al., 2002; Weinsier et al., 1992). As such, the relative RMR threshold that indicates metabolic suppression may differ in athletes with differing physique characteristics. The use of a RMR ratio threshold to indicate metabolic suppression in male athletes also needs to be considered, as the implemented thresholds were validated in women and not men (Strock et al., 2020), and therefore may not be appropriate for male athletes. It is unknown if LEA was a contributor to the cases where athletes presented with a suppressed RMR measurement. This is further complicated by the time period of LEA that results in a suppressed RMR being unknown, as well as the time course of a suppressed RMR to recover following the restoration of EA being unknown. Many athletes in this study periodically presented with a suppressed RMR measurement rather than having a persistently suppressed RMR measurement for all five conditions, and it seems unlikely that

the ~8 day washout period between conditions would be sufficient for an athlete to develop a state of LEA that would result in metabolic suppression or increase EA to a degree that would restore a previously suppressed RMR. There were also no apparent differences in the prevalence of indicators of LEA in athletes with and without a suppressed RMR. For instance, two male athletes had low total or free testosterone across all conditions with testosterone levels falling in a range indicative of a severe primary indicator on several occasions, but only one of these athletes presented with a suppressed RMR measurement. Similarly, a female athlete presented with a suppressed RMR for four of the five conditions, but never presented with a primary, secondary, or potential indicators of LEA. The majority of LEA indicators were unaffected by the 24-hour conditions implemented in this study. However, cortisol and insulin concentrations were affected by EI and EEE in the preceding 24 hours, suggesting that prior diet and exercise may contribute to variability in their measurements. As such, diet and exercise in the 24 hours prior to measuring these markers should be controlled to avoid erroneous measurements. Notably, the high prevalence of elevated cortisol levels seen in female athletes in this study is likely due to the use of COC, which are known to increase both cortisol binding globulin and total cortisol levels (Özcan et al., 2023). Indicators of LEA should be used as per the updated REDs CAT2 when being used to assess for risk of REDs (Stellingwerff et al., 2023) and accordingly RMR should be used with caution to diagnose LEA until Best Practice Protocols are fully developed to increase the precision of RMR measurements.

While the diet and exercise manipulations did not impact RMR measurements, differences in body composition estimates across conditions were seen. For both male and female athletes, DXA estimates of whole-body LBM and FFM were greater following the GEA_{rest} and HEA_{ex} conditions compared to the LEA_{rest} and LEA_{ex} conditions, whereas FM estimates were unaffected by the conditions. DXA body composition estimates can be influenced by glycogen

levels. For instance, an increase in estimates of LBM following a 48-hour glycogen loading protocol (carbohydrate intake ~12 $g \cdot kg^{-1} \cdot day^{-1}$) has been observed in male cyclists (Bone et al., 2017) and after three days of a high carbohydrate diet (~8 $g \cdot kg^{-1} \cdot day^{-1}$) in physically active males (Rouillier et al., 2015). The conditions of LEA (LEA_{rest} and LEA_{ex}) may have resulted in reduced muscle glycogen stores, as has been seen by others under conditions of LEA (3 days of ~20 kcal·kg $FFM^{-1} \cdot day^{-1}$) (Kojima et al., 2020), but the levels of carbohydrate intake for the GEA_{rest} and HEA_{ex} (~7.5-8 $g \cdot kg^{-1}$ over 24 hours) were unlikely to have resulted in glycogen supercompensation, as glycogen loading protocols typically involve a carbohydrate intake of ~10-12 $g \cdot kg^{-1} \cdot day^{-1}$for 36-48 hours (Burke et al., 2011). Additionally, previous reports of increases in estimates of LBM following glycogen loading protocols also found increases in leg (Bone et al., 2017) and appendicular LBM (Rouillier et al., 2015) whereas we found no differences in body composition estimates of legs or arms across conditions, but rather just differences in the estimates of trunk LBM and FFM across conditions (see Figure 7.2). Reduction in FFM and LBM, particularly trunk estimates, have been reported with dehydration (Going et al., 1993; Rodriguez-Sanchez & Galloway, 2015; Toomey et al., 2017). Based on morning USG, the only observed difference in hydration was between the LEA_{rest} and HEA_{ex} with a more dehydrated state in the HEA_{ex} condition. If differences in hydration were responsible for the observed differences in body composition estimates, you would expect to see a reduced FFM and LBM with the HEA_{ex} rather than the greater estimates that we observed. Another possibility is that the observed differences in body composition across conditions was due to differing volumes of food consumed the day prior as alterations in DXA scan body composition estimates have been reported when they occur following food and/or fluid consumption rather than in a fasted state (Nana et al., 2012; Ong et al., 2022a; Tinsley et al., 2017). Because of the error introduced by prior food and fluid consumption on DXA body composition estimates, it is recommended that DXA scans occur in an overnight fasted state

(Slater, Townsend, et al., 2023). However, our results demonstrate the importance of measurements not only occurring in an overnight fasted state, but also that diet and exercise should also be controlled in the 24 hours prior to a DXA scan that is being used to estimate body composition. This has indirect implications for RMR guidelines, as DXA scans are often performed alongside RMR measurements to help interpret findings.

Strengths of this study include the strict dietary and exercise control with all food being pre-portioned and provided to participants, and exercise sessions being supervised. However, water intake was ad libitum rather than controlled, and the absence of tracking this prevents a determination of differences in the fluid volumes consumed across conditions. Nevertheless, hydration status on presentation for each DXA scan was assessed by USG. Extrapolation of these findings is further limited to cycling, and as highlighted above, different results may be seen with other forms of exercise.

7.6 Conclusion

In conclusion, we did not find evidence that EI and/or exercise in the 24 hours prior to testing contributes to variations in RMR measurements. This suggests that the TEF and EPOC did not persist into next day RMR measurement, nor did energy flux create variation in RMR measurements. However, differences in body mass and DXA body composition estimates of LBM and FFM were seen between conditions. While EI and moderate intensity exercise to which an athlete is accustomed is unlikely to contribute to erroneous RMR measurements, diet and exercise should be controlled prior to scenarios in which optimal precision of DXA body composition estimates is required.

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Conflict of Interesting

The authors declare no relevant conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

7.7 Interlinking chapter

The third study of this thesis demonstrated that variations in EI are unlikely to create artifacts in next-day RMR measurements due to residual TEF. Likewise, exercise had no effect on RMR measurements that occurred 12 hours post-exercise, suggesting that EPOC did not carry over and create artifacts in next-day RMR measurements. As such, diet does not need to be standardised, and athletes can engage in exercise to which they are accustomed the day prior to a RMR measurement.

The final study of this thesis examined the use of RMR measurements within the Australian high-performance sport environment. Using a qualitative research design, we assessed for barriers and enablers to measuring RMR in the high-performance sport environment.

8 Study 4: Barriers and Enablers to Measuring Resting Metabolic Rate in the High-Performance Sporting System: A Qualitative Exploratory Study.

Publication statement:

This chapter is comprised of the following paper that is in review and has undergone one round of revisions in the Journal of Sports Sciences.

Kuikman, M.A., McKay, A.K.A., Brown, H., Townsend, N., McCormick, R., Morabito, A., Pichshev, N., Slater, G., and Burke, L.M. (2024). Barriers and enablers to measuring resting metabolic rate in the high-performance sporting system: A qualitative exploratory study.

The chapter does not differ from the submitted paper apart from tables, figures and references, which have been renumbered to maintain consistency within the thesis.

8.1 Abstract

Measurements of resting metabolic rate (RMR) may be undertaken for dietary planning purposes or to assess for low energy availability (LEA). This study investigated barriers and enablers to measuring RMR in real-world, high-performance sport environments. Twelve interviews were conducted with technicians (n=6 dietitians, n=6 physiologists) employed across six National Institute Networks, two National Sporting Organisations and one professional sporting code. RMR was predominantly measured to screen for LEA with measurements only occurring in a few instances for dietary planning purposes. Data was thematically analysed with six main themes identified. Barriers included lack of confidence in measuring RMR, burden of measurement on athlete and technician, confusion over measurement responsibility, and scepticism in RMR measurements as an indicator of LEA. Subthemes that contributed to scepticism included: the RMR thresholds used to indicate LEA, unanswered research questions, and measurement errors introduced by athlete presentation, testing equipment and/or environment. Enablers to use of RMR measurements included perceived value of RMR measurements as a "piece of the puzzle" when assessing for LEA and its use as a longitudinal measure. Best practice guidelines for RMR measurements in athletic cohorts must consider these barriers and enablers as they highlight unique characteristics of athletes and their environment.

8.2 Introduction

Basal metabolic rate (BMR) represents the minimal energy cost of living (Hulbert & Else, 2004) and makes up one of the components of total daily energy requirements alongside the thermic effect of food, exercise activity thermogenesis, and non-exercise activity thermogenesis (Trexler et al., 2014). Metabolic rate can be measured using indirect calorimetry by measuring the amount of oxygen consumed and carbon dioxide produced through respiratory gases (Haugen et al., 2007) and then using the modified Weir equation to determine energy expenditure (Weir, 1949). The measurement of metabolic rate in an outpatient setting, with the individual sleeping outside of the laboratory and commuting to the laboratory for testing in a fasted and rested state, is known as resting metabolic rate (RMR) (Manore, 2021). Measurements of RMR are typically higher than BMR, but similar results are seen when there is an adequate period of rest prior to the RMR measurement (Bone & Burke, 2018; Turley et al., 1993). As measuring metabolic rate in outpatient conditions is more common, and used within the Australian high-performance sport system, the terminology of RMR will be used throughout the remainder of this paper.

Knowledge of an athlete's RMR has many practical applications for those involved in athlete care. This includes providing valuable information on an athlete's energy requirements given RMR makes up one of the components of total daily energy expenditure (Trexler et al., 2014) which can help guide support staff who give nutrition advice and plan the diet of an athlete. While an athlete's RMR can be estimated from predictive equations, most have excluded athletes in their analysis or only included a small number of athletes (Schofield et al., 2019). Investigations of the application of these predictive equations to athletic populations have found that they tend to underestimate an athlete's RMR (Carlsohn et al., 2011; Jagim et al.,

2018; Thompson & Manore, 1996). As such, when an accurate RMR value is required, laboratory testing using indirect calorimetry is recommended.

A more novel use of RMR measurements is as a diagnostic tool for low energy availability (LEA) (Sterringer & Larson-Meyer, 2022). LEA occurs when there is a mismatch between energy intake and exercise energy expenditure, leaving inadequate energy to support bodily function (Mountjoy et al., 2023). LEA may lead to Relative Energy Deficiency in Sport (REDs), a syndrome of impaired physiological function with various health and performance consequences (Mountjoy et al., 2023). One such consequence includes an impaired metabolism resulting in a suppressed RMR (Mountjoy et al., 2023). This is assessed by comparing the ratio of a laboratory measurement of an athlete's RMR using indirect calorimetry against predicted RMR using one of the widely available equations, or by an athlete's fat free mass (FFM) to a theoretical classification value (Stellingwerff et al., 2023). However, within the International Olympic Committee (IOC) REDs clinical assessment tool (CAT2), which relies on the measurement of primary and secondary outcome indicators to determine the risk and/or severity of LEA, measurements of RMR have been listed as "emerging" due to concerns regarding the specificity and sensitivity of this measurement (Stellingwerff et al., 2023). This represents a novel use of RMR measurements within high-performance sport setting.

Despite the aforementioned use of RMR measurements within high-performance sport, current Best Practice Guidelines for RMR measurements were developed for non-athletic populations (Compher et al., 2006; Fullmer et al., 2015). For efficacy within the sporting context, Best Practice Guidelines that consider the unique characteristics of athletes and their environment are needed as this differs to non-athletic populations. Additional considerations are also needed when RMR measurements are being used to screen for LEA in athletic cohorts in order to

address the specificity and sensitivity concerns that hinder this measurement as an indicator of LEA (Stellingwerff et al., 2023). Therefore, to inform guideline development, an understanding of contextual factors in a real-world setting is needed. As such, the aim of this study was to investigate barriers and enablers to measuring RMR in the real-world high-performance sport environment, and to provide strategies to address identified barriers.

8.3 Methods

8.3.1 Study design

A qualitative research design was used to investigate use of RMR measurement in a real-world high-performance sport environment and identify potential barriers and enablers to use to inform future guideline development. A qualitative approach was taken to explore technicians' perceptions and thoughts relating to RMR measurements. The study was approved by the Human Ethics Research Committee at Australian Catholic University (2023-3380E).

8.3.2 Research philosophy

A relativist ontology and constructivist epistemology methodological approach were adopted, which takes the perspective that multiple realities can exist (Willis, 2007).

8.3.3 Participants

Inclusion criteria included being employed by a National Institute Network (NIN), National Sport Organisation (NSO) or professional sporting code in Australia and measuring the RMR of athletes either in the past or presently as part of this role.

8.3.4 Recruitment

Individuals at NINs, NSOs or professional sporting codes throughout Australia were contacted via e-mail, phone-call or in-person discussions from a member of the research team with details regarding the study. Recruitment was facilitated by established connections between members of the research team, and the NINs, NSOs and professional sporting codes. Initially, individuals at seven of the NINs were invited to participate, as well as eleven individuals from NSOs and

professional sporting codes throughout Australia. In situations where the individual contacted did not conduct RMR measurements, there was a request made for the contact details of the technician conducting the RMR measurement within that NSO or professional sporting code. Finally, at the end of each interview, technicians were asked if they were aware of anyone else measuring RMR in athletic cohorts in Australia. Attempts were made to interview at least one technician at each State and Territory Institute, NSO and professional sporting code in Australia that measures RMR to ensure comprehensive viewpoints and experiences. All technicians provided written informed consent to participate and have interviews recorded and automatically transcribed.

8.3.5 Data collection

For technicians that consented to proceed with the study, an in-depth interview was scheduled with one member of the research team (MK) via a video call. These interviews were recorded and transcribed verbatim. The semi-structured interview schedule was developed by the research team (experts in the field), acknowledging the need for adapting questions as interviews proceeded. The interview questions were pilot tested, and feedback was used to refine the questions resulting in twenty-six questions with an average interview duration of 48 minutes.

8.3.6 Data analysis

Reflexive thematic analysis was completed to enable full data exploration and familiarisation. An inductive approach was taken, allowing researchers to identify themes from the data. The first author familiarised herself with the data by listening to interview audio-recordings following each transcript to ensure accuracy of automatic transcription and reading each transcript at least twice. Written transcripts were uploaded into NVivo 12 (QSR International, Melbourne, Australia) and initial codes generated inductively by the authors (MK and HB) and assigned to relevant text segments. Codes were then collated into major themes and sub themes

by the authors, and these themes were then reviewed and discussed with another member of the research team (HB). Any discrepancies in coding were resolved through consensus discussion among the research team. In order to provide strategies to address identified barriers and provide practical consideration for measuring RMR in the high-performance sport system, themes were mapped to the Capability, Opportunity, Motivation – Behaviour (COM-B) model and theoretical constructs of Theoretical Domains Framework (TDF) (Michie et al., 2011). The COM-B model is a framework for understanding behaviour (Michie et al., 2011). Capability refers to the technician's psychological and physical capacity to measure RMR, including having the necessary knowledge and skills, motivation refers to the brain processes that energise and direct behaviour, and opportunity is defined as factors that lie outside of the technician that make it possible to measure RMR (Michie et al., 2011). To provide further information on behaviour, the COM-B model can be mapped to the TDF, which includes 14 domains: knowledge; skills; social/professional role and identity; beliefs about capabilities; optimism; beliefs about consequences; reinforcement; intentions; goals; memory, attention and decision processes; environmental context and resources; social influences; emotions; and behavioural regulation (Cane et al., 2012).

8.3.7 Trustworthiness

Several methods were employed to enhance trustworthiness. Peer debriefing and investigator triangulation (MK and HB) were used to confirm findings and allow for different perspectives (Creswell & Miller, 2000; Spall & Austin, 1998.).

8.4 Results

Twelve interviews were conducted with technicians (n=6 dietitians and n= 6 physiologists) employed across six NINs, two NSOs and one professional sport code. Of those interviewed, two technicians no longer measured RMR in athletes, although this measurement was previously conducted. Two technicians used the first-principle (Douglas bag) method to

measure RMR with the remaining technicians using a metabolic cart to measure RMR. RMR measurements predominantly occurred to assess for LEA with all technicians indicating that this was the reason for measurement referral. In addition to screening for LEA, two technicians also noted the use of RMR measurements for dietary planning purposes.

8.4.1 Thematic analysis overview

The results presented here highlight the themes identified, which were then grouped according to whether they were barriers or enablers to measuring RMR as an indicator of LEA.

8.4.2 Barriers to using RMR measurements as an indicator of LEA

8.4.2.1 Lack of confidence in measuring RMR

A lack of confidence in measuring RMR became evident when interviewing technicians. While technicians met the standards regarding qualifications and accreditation, it was noted that technicians were often expected to measure RMR without specific training in this measurement. Notably, a formalised training process to measure RMR was reported only by the two technicians using the Douglas bag method to measure RMR. Two technicians reported that they were requested to measure an athlete's RMR when beginning a new role despite no experience and specific training in measuring RMR:

> Technician 6: "I wasn't trained… I started this job… and basically my first week got asked to do one by the dietitian and just like read the instructions and did it."

> Technician 10: "It's like I was thrown in, and also at a time where I was just new to a job… I didn't really know what the common practices were."

8.4.2.2 Burden of RMR measurements

Many technicians expressed the burden of measuring RMR both for themselves and for athletes. Burden for technicians included the time required to measure RMR with an already heavy workload. Perceived burden for athletes included the time associated with the test that may interfere with training, the requirement to come to the laboratory early in the morning in

an overnight fasted state, and measurement protocols that made the test uncomfortable for athletes. Perceived sources of athlete discomfort included the mouthpiece and nose clip, and requirement to lay supine for 1 hour.

> Technician 6: “Have you done everything else before? We set up, it's an hour. Measurements, an hour. Pack up, 30 minutes. like 2.5 hours. That's a significant resource. Especially like a gold medallist. You really wanna know? In my opinion, the other things need to come first [in reference to other indicators of LEA]”

The burden of RMR measurements contributed to technicians not frequently undertaking assessments:

> Technician 1: “The thought of devoting time to the RMR again, when it's a test we don't use a lot is just something I… don't have the time and the energy for.”
>
> Technician 3: “We don't do it very often because of the burden on the athlete and on the staff having to measure it.”

8.4.2.3 Confusion over measurement responsibility

All technicians reported that dietitians referred athletes for RMR measurements for LEA screening purposes. As noted above, the use of RMR measurements for other purposes was only noted in a few instances. There was disagreement regarding whether dietitians or physiologists should be responsible for measuring RMR as well as the need for collaboration across professions.

> Technician 10: “What I think is important for a dietitian, is to do the test themselves because they're interpreting the data, so that they can see the variability of the tests firsthand and understand and interpret the information because they've measured it.”

> Technician 2: “almost all of the physiologists … said that they would not measure RMR because of the problems with the protocol and the lack of consistency and accuracy in the measures… I don't know if it's because dietitians want the measure and it would be the

physiologists running it, so the dietitians have value in the outcome, and the physiologists are unsure about the process and they don't have anything to get out of it."

Technician 8: "It would be good to get more collaboration across nutrition and physiology in terms of an agreed direction and how we engage together in this space going forward."

8.4.2.4 Scepticism in RMR measurement as an indicator of LEA

There was a general sense of scepticism in RMR measurements as an indicator of LEA among interviewed technicians:

Technician 3: "I don't really see a huge amount of value in it. I want to look at other indicators [of LEA] before requesting a RMR."

Technician 4: "So the reason that it's now more targeted is a lot to do with our confidence in the answers and the caution that I believe is needed around RMR being the be all and end all."

Technician 6: "In my experience it's either just provided a confirmation of something we already knew, or we get a result that we go, "Ohh actually it's just a point in time and whatever they did yesterday might have affected it." And… go back to whatever we thought before we did the RMR, so I'm like, what's the point?"

Technician 11: "I haven't used it simply because I don't trust the data enough that it'll show something useful."

Technician 8: "As a regular screening tool [for LEA], I'm still not sold."

Four sub-themes were noted to contribute to scepticism in RMR measurement as an indicator of LEA (see Table 8.1). This included lack of evidence to support the RMR thresholds used to indicate LEA, unanswered research questions, and error introduced in the RMR measurement due to athlete state during the test, and the equipment and facilities used to measure RMR.

Table 8.1. Sources of scepticism in RMR measurements.

Sources of scepticism	Sample quotation
RMR threshold	Technician 2: “the measured to predicted 0.9 threshold didn't make a whole lot of sense because you're using an equation that's estimating RMR compared to an actual measured RMR, and you're saying the estimating is better… and you're judging your measured RMR… to that.”
	Technician 7: “I think the main one we've always come back to is… a better understanding of where athletes could and should sit and the variance”
	Technician 9: “You never can know what someone's RMR is supposed to be unless you've got that historical understanding of them… and we've got a large number of Pacific Islander, Maori indigenous athletes… My observation of a lot of these athletes is they eat significantly less than what I would expect them to eat for the training that they're doing and to support the muscle mass that they have, and so it does make me wonder, is there just something different in their Physiology?... It just adds that other element of complexity because we don't really know what normal metabolism is in this population exactly.”
Athlete state	Technician 1: “You know the noise is probably what that athlete has done training wise the day before.”
	Technician 2: “We have camps come in of under 21 athletes who've never been to the X that arrived into ressies and we get them up early in the morning, they have to try and find this building and get greeted by someone they didn't know and taken through to the blood taking area with all these like biohazardous waste bags and syringes and then you give them all these instructions and then they go away and you just think, oh, that's really not a good practice.”

	Technician 6: “we potentially measure someone when they're in the height of a camp… and then later we'll measure them post like a competition or something, and they've been off for two weeks and you're obviously gonna get very different results.”
	Technician 7: “The noise in the athlete presentation on the day, be it their sleep they might have had the night before, the training or the food they might have had the day before. I think probably sleep and stress levels… play a bit more of a role.”
	Technician 8: “The biggest challenge in our environment is that people have to drive here. They become alert, driving their cars in traffic… and then trying to get them back to a true resting state is always a challenge.”
Equipment and facilities	Technician 1: “I actually feel that the hood may have made the RMR appear actually lower than what it really was. Because a lot of the results I was getting back, majority of them would have been indicative of low energy availability…. it's just this Dome thing on top of you and you're just lying on a massage table with a pillow under your head that for some was actually quite comfortable and relaxing and may have actually I think sometimes resulted in a lower RMR than what potentially is the case.”
	Technician 3: “We don't have the greatest setup here for it.. it's like an old kind of doctor's bed… some of the athletes are too long for it… they can't get comfortable and it's just in a random room.”
	Technician 4: “I think there is noise in the un-comfort, so the mouthpiece, the nose clip… that adds a lot of noise for us”
	Technician 7: “I think it is that compounding error of the DXA [contributing to variability in RMR as an indicator of LEA]”
	Technician 9: “I think it's really the mouthpiece that's the issue.”

	Technician 10: “You could basically get quite a few different numbers within the testing window… so I think it's often difficult to know that that's the correct number to use because there could be multiple time points where we get multiple different numbers.”
	Technician 12: “Well maybe that's actually a DXA thing rather than a total metabolic output measurement differences… different brands can give you different values, and so just plugging in the muscle mass value from whatever DXA that they've got available to them.”
Unanswered question	Technician 1: “Is it just adaptable and that's showing up as low RMR and they're in an adaptable low energy availability state? or is this part of a chronic or problematic low energy availability that's contributing to a low RMR?”
	Technician 2: “I think probably the biggest need is some more research around like the timelines for change in RMR. Like does it become acutely low from short periods of energy restriction and is that actually low energy availability or is that just low energy intake for a few days which you know is different? and how quickly does it recover?... and how much does training affect it?”
	Technician 4: “I think there's still a lot more questions that we need answers for us to feel super comfortable”
	Technician 9: “It's one of those things that you think it's going to be useful and then you just realise how little we know and understand about this and it just, you know, opens up a big catalogue of questions.”

DXA, dual-energy X-ray absorptiometry; LEA, low energy availability; RMR, resting metabolic rate.

Unanswered research questions identified by technicians that contributed to scepticism are highlighted in table 8.2.

Table 8.2. Questions of high research priority that emerged during the interview process.

How does RMR ratio and relative RMR thresholds indicative of LEA risk change with 1) athlete calibre 2) athlete type (power, endurance, team- sport etc.) 3) age 4) sex 5) ethnicity 6) physique?
What is the effect of training status and/or intensified training on RMR independent of EA? For instance, do high training loads with adequate EA cause a reduction in RMR?
What is the effect of boredom, discomfort and/or anxiety on RMR measurements? For instance, are different results seen when testing occurs on a comfortable bed vs. a small massage bed? Does fidgeting during measurements lead to more variable estimates?
What is the time frame of change in RMR in response to change in EA? For instance, what duration and magnitude of LEA results in a suppressed RMR? Does this differ with biological factors, such as age or sex?
How fast does a suppressed RMR recover in response to a period of increased energy intake? For instance, does a suppressed RMR recover before or after other biological systems impacted by LEA in situations of increased energy intake? Could recovery of a suppressed RMR be used as an index of adequacy of an intervention?

EA, energy availability; LEA, low energy availability; RMR, resting metabolic rate.

8.4.3 Enablers to using RMR measurements

8.4.3.1 Perceived value of RMR measurements as a "piece of the LEA assessment puzzle"

Measurements of RMR were expressed as being valuable when taken alongside other LEA indicators as it provided another "piece of the puzzle."

> Technician 1: "It can be a good piece… one piece of the puzzle in conjunction with a lot of other information."

Technician 2: “I think you just have to be careful to look at the whole clinical picture and not just take the RMR measurement out of context and use it on its own.”

Technician 3: “I also don't think it should be used purely as indicator for low energy availability, like the only thing [measured]. It should be used as one of the tools that you're looking at, not as the one indicator.”

Technician 5: “RMR can be used as an additional piece to the puzzle, not as like a final say, it's just like an additional bit of information.”

Technician 7: “It has to be in the right scenario, the right context, with other information collected as well.”

8.4.3.2 Value of RMR measurements as a longitudinal measure

Technicians identified the importance of measuring an athlete’s RMR repeatedly rather than as a one-off measure as this allowed a determination of what an athlete’s “normal” RMR is, and to track its change over time. Longitudinal RMR measurements also served as an educational tool to reinforce interventions.

Technician 2: “I've got multiple examples of people who had a lower RMR and you give them the new nutrition intervention and they have a really positive change in some feature of themselves. So it might be they’ve increased LBM [lean body mass], it could be their training resilience is a lot better, that bone mineral density increased, like there's really clear clinical outcomes when you make a change.”

Technician 3: “I think maybe not so much as a once off measure, but as a repeated measure, so you can see if there's trends… because we are all different and we will all have our different RMRs.”

Technician 4: “If I've got a new athlete and I do it and I have one data point… I would want a couple more data points to get a sense of what's their normal.”

Technician 7: “It wasn't just on the verge of the cut off, it was so low. And then after the period of changing things, be it a reduction in training, increase in intake over a period of time, and

then it was so clearly in the good… it sort of just helped support the message we're already using."

8.4.4 Practical considerations for measuring RMR in the high-performance sport system

Barriers and enablers mapped to the COM-B model and TDF with recommended strategies are summarised in Table 8.3.

Table 8.3. Barriers and enablers to measuring RMR in the high-performance sport systems and corresponding strategies to address mapped to the Capability, Opportunity, Motivation- Behaviour (COM-B) model and theoretical constructs of the Theoretical Domains Framework (TDF).

Barrier/Enabler	Evidence	COM-B	TDF	Strategy
Lack of confidence in measuring RMR	"I wasn't trained… I started this job… and basically my first week got asked to do one by the dietitian and just like read the instructions and did it."	Capability Motivation	Knowledge and skills Self-confidence	Mandate formalised training and educational opportunities on how to measure RMR
Burden of measurement	"We don't do it very often because of the burden on the athlete and on the staff having to measure it."	Motivation Opportunity	Beliefs Environmental resources and stressors	Implement practices and protocols that maximise measurement comfort, including checking and challenging current measurement protocols for duration of rest, familiarisation and data collection Schedule measurement as close to natural wake time of athlete as possible
Confusion over measurement responsibility	"It would be good to get more collaboration across nutrition and physiology in terms of an agreed	Motivation	Professional role	Clearly stipulate who is responsible for measuring RMR at each institution or organisation and the process of RMR measurement referral

	direction and how we engage together in this space going forward.”			Provide education on the use of RMR measurements as an LEA indicator for non-referring practitioners that measure RMR
Scepticism in RMR thresholds	“The measured to predicted 0.9 threshold didn't make a whole lot of sense”	Motivation	Beliefs	Provide education on the use of and limitations of RMR thresholds Measure RMR repeatedly in an athlete to measure changes over time Build a normative RMR data base that accounts for factors that create variability in RMR measurements
Unanswered questions	“It's one of those things that you think it's going to be useful and then you just realise how little we know and understand about this and it just, you know, opens up a big catalogue of questions.”	Motivation	Beliefs	Provide answer to questions where they exist Ongoing research needed to address unanswered questions

Error due to athlete state	"You know the noise is probably what that athlete has done training wise the day before."	Motivation	Beliefs Outcome expectancies	Provide clear guidelines on athlete standardisation requirements prior to RMR measurements
Error due to facilities and equipment	"I think there is noise in the un-comfort, so the mouthpiece, the nose clip… that adds a lot of noise for us"	Motivation	Beliefs Outcome expectancies	Provide clear guidelines specific to athletic populations on requirements for facilities, equipment and environment where RMR is measured that maximises athlete comfort
RMR measurements as a valuable piece of the puzzle	"It can be a good piece… one piece of the puzzle in conjunction with a lot of other information."	Motivation	Beliefs Outcome expectancies	Measure primary and secondary indicators of LEA alongside RMR measurements
RMR measurements valuable when measured longitudinally	"If I've got a new athlete and I do it and I have one data point… I would want a couple more data points to get a sense of what's their normal."	Motivation	Beliefs Outcome expectancies	Measure RMR repeatedly in an athlete in order to assess changes over time, and in response to an intervention

COM-B, Capability, Opportunity, Motivation- Behaviour; LEA, low energy availability; RMR, resting metabolic rate; TDF, Theoretical Domains Framework.

8.5 Discussion

Various barriers and enablers to the use of RMR measurements in the high-performance sport system emerged during the interview process. Notably, RMR measurements almost exclusively occurred for LEA screening purposes. The identified barriers allowed for the identification of strategies that must be considered when measuring RMR in the high-performance sport system. These must also be considered when developing Best Practice Guidelines for measuring RMR as they provide important information on contextual factors unique to athletes and their environment, and as such, will increase acceptance by technicians working within the high-performance sport system.

Lack of confidence in measuring RMR emerged as a barrier to RMR measurements by technicians working within the high-performance sport system. This barrier demonstrates the need for a formalised training process to measure RMR to increase technician confidence in their ability to measure RMR and interpret findings. The importance of formalised training and technician experience has been highlighted by other measurements that occur within the high-performance sport system, such as the use of dual energy x-ray absorptiometry (DXA) scans to estimate an athlete's body composition (Persson et al., 2019). The significance of training was mentioned by a technician in discussion of how RMR measurements could be improved, who stated: "*definitely more training and maybe more of a dedicated role of somebody who does RMR measurements*." The requirement to undergo training will also help to clarify who is responsible for measuring an athlete's RMR within an institution or organisation. To further ensure collaboration across differing professions, institutions and organisations should clearly outline the RMR measurement referral process. Technicians measuring RMR, but not providing the referral, should also be educated on what RMR measurements are used for, so that they perceive value in the measurement.

Another barrier that must be addressed for RMR measurements guidelines to be accepted and implemented within the high-performance sport system is the burden of the measurement for technicians. A single RMR measurement typically involves a period of 10 minutes of rest, 15 minutes of familiarisation, and then 20-30 minutes of data collection (Bone & Burke, 2018). Yet, the burden associated with the time would likely be reduced if RMR measurements were seen as more valuable. This was highlighted by a technician who reported value in RMR measurements, *"or I wouldn't do it. It's a pain in the backside to do."* However, it's also worth mentioning that the protocol used for RMR measurements needs to be checked and challenged as a shorter time frame may offer grater time efficiencies without compromising measurement precision. Technicians also perceived a burden of the measurement for the athlete undergoing the measurement. For this to be addressed, there is a need to engage with athletes firsthand to understand their experience with this measurement and factors that may be contributing to measurement burden.

An athlete's state during the RMR measurement as well as the equipment and facilities used to measure RMR were a perceived source of scepticism by introducing error in the measurement. For instance, many technicians expressed concern that discomfort with the mouthpiece and nose clip could contribute to error in RMR measurements. On the other hand, the one technician that used a ventilated hood when measuring RMR felt that this resulted in an artificially low RMR. Although not a consistent finding (Segal, 1987), RMR measurements have been reported to be lower with a ventilated hood than mouthpiece (Roffey et al., 2006). Until the extent of technical variability from differing equipment and facilities is determined, a uniform approach that prioritises athlete comfort should be taken across the sport system when measuring RMR.

Lack of athlete standardisation prior to the RMR measurement was further perceived to be a source of error that contributed to technician's scepticism. In particular, many technicians expressed concerns that an athlete's training was contributing to error in RMR measurements. A sufficient period is needed between exercise and a RMR measurement to avoid an artificially elevated measurement due to the increase in metabolic rate that persists with the cessation of exercise (Børsheim & Bahr, 2003). The Evidence Analysis Library on RMR measurements was not able to provide guidelines on the duration that individuals should refrain from exercise prior to a RMR measurement due to lack of research (Fullmer et al., 2015). While work from our group suggests that endurance exercise does not carry over and create artefacts in next-day RMR measurements (Kuikman, Smith, et al., 2024), there is evidence that resistance exercise may lead to artificially increased RMR measurements (Dolezal et al., 2000; Gillette et al., 1994; Hackney et al., 2008; Jamurtas et al., 2004; Melby et al., 1993; Paoli et al., 2012; Osterberg et al., 2000; Schuenke et al., 2002). As such, Best Practice Guidelines for RMR measurements should stipulate that athletes can engage in endurance exercise to which they are accustomed the day prior to a RMR measurement. However, further research is needed looking at the carry-over effect of resistance training or exercise that damages muscle on next day RMR measurements. For instance, fluctuations in RMR have been reported across match week in soccer players (Carter et al., 2023) and rugby union players with an increased RMR following game day thought to be from muscle damage (Hudson et al., 2020). As taking a day off from training may not be a realistic option for the high-performance athlete, the phase of an athlete's training cycle should be recorded and considered when interpreting results and changes over time.

The RMR thresholds used to indicate a suppressed RMR also emerged as a source of scepticism when using RMR measurements as an indicator of LEA. This scepticism is appropriate as the

IOC REDs CAT2 recommends against using these thresholds as an indicator of LEA at this time as further research is needed to support its use (Stellingwerff et al., 2023). Of note is the origin of the thresholds commonly used to indicate a state of LEA. The study that validated the RMR ratio thresholds used a ventilated hood when measuring RMR with study participants including women who were only required to exercise ≥2 hours/week (Strock et al., 2020). These thresholds may change with differing gas collection devices (i.e. mouthpiece instead of hood) and may not be appropriate for men or the nuanced presentations of athletes, who may present with disproportionate LBM and likely engage in larger volumes of exercise. The origin of a relative RMR threshold of 30 kcal·kg FFM^{-1}·day^{-1} indicating a state of LEA is unclear with various papers (Loucks et al., 2011; Melin et al., 2015; Thompson et al., 1993; Westerterp, 2003) often referenced to support this threshold, but none appropriately supporting this cut-off. Additionally, care needs to be taken with use of the relative RMR threshold as the RMR to FFM ratio is reduced with increased FFM due to an increased proportion of FFM as low metabolic rate tissue (Heymsfield et al., 2002; Weinsier et al., 1992). As such, both the RMR ratio and relative RMR thresholds may not be universally appropriate for all athletes, and this represents an area of high research priority.

Despite the scepticism of the RMR thresholds, enablers to RMR measurements included being perceived as a valuable assessment when measured longitudinally and as a piece of the puzzle for LEA assessment. Notably, the barrier of scepticism in RMR thresholds can be overcome by relying on the enabler of measuring RMR longitudinally as this allows for the assessment of trends rather than relying on RMR thresholds. However, test-retest reliability at testing locations must also be determined in order to determine what is considered meaningful change in RMR over time. The perceived value of RMR measurements as a piece of LEA assessment puzzle highlights the importance of RMR measurements occurring alongside other indicators

of LEA. This again aligns with the 2023 REDs CAT2, which cautions against using RMR measurements as an indicator of LEA at this time (Stellingwerff et al., 2023). As such, when RMR measurements are being used to assess for an athlete's risk of REDs, primary and secondary indicators of LEA should be taken alongside RMR measurements (Stellingwerff et al., 2023), so that this information can be interpreted as a whole.

Of those interviewed, one technician stood out as having successfully implemented RMR measurements as a screening tool for an athlete's energy status within the sporting environment. Themes were noted that contributed to this successful implementation. This technician knew the athletes within the program well, completed formative work to develop a protocol for measuring RMR, and took an athlete centred approach when measuring RMR. For instance, this technician worked with the same athletes across multiple Olympic cycles. This enabled longitudinal measurements of RMR over an athlete's career rather than just as a one-off measurement to screen for LEA. This technician had further evolved the practices and protocols used to measure RMR over time. At one point in the discussion of practices and protocols, this technician stated: "*I've tried pretty much everything*." The evolved protocol was also athlete-centred and reduced the burden of the measurement for the athletes within the program. For instance, this technician measured athlete's RMR in a location close to their daily training environment, so that the testing environment was familiar to athletes, and they could commence training as soon as the measurement was complete. This served as a powerful example of the successful implementation of RMR measurements within a real-life sporting context. A summary of recommended strategies for consideration when developing Best Practice Guidelines for RMR measurements in athletic cohorts can be found in Table 8.3. Incorporating these strategies into guidelines for RMR measurements in athletic cohorts may

increase the effectiveness and acceptance by technicians working with high-performance athletes.

8.6 Conclusion

Measurements of RMR within the high-performance sporting context predominantly occurred to screen for LEA. Within this environment, there were various barriers and enablers to measuring RMR. These should be addressed when developing Best Practice Guidelines for RMR measurements in athletic cohorts given they consider contextual factors that are unique to the athlete and their environment. There is a further need to test implementation strategies for these guidelines, such as educational strategies, for successful implementation.

9 Discussion and Conclusion

While the measurement of RMR has traditionally been used to determine an athlete's energy requirements, it has gained traction more recently as a screening tool for LEA. However, the REDs CAT2 considers RMR assessment as a "potential" indicator of LEA – defined as purposefully vague in quantification, pending further research to quantify parameters and cut-offs more accurately – due to poor measurement validity and reliability (Stellingwerff et al., 2023). Multiple factors are likely to contribute to variability and/or artifacts in RMR measurements. Investigation of these factors is needed to decide if RMR measurements are sufficiently valid for use as a primary or secondary indicator of LEA, or if disproved, removed as a potential indicator of LEA within the REDs CAT2. The aim of this thesis was to investigate factors contributing to variability or error in RMR measurements by answering the following questions through a series of studies:

1. Study 1: Do changes in sex hormones across the MC or with HC usage in female athletes introduce biological variability in RMR measurements?
2. Study 2: What is the effect of low altitude on RMR, and does this change with time exposed to altitude? Is the effect of LEA on RMR moderated by altitude exposure?
3. Study 3: Do diet and exercise need to be standardised in the 24 hours prior to a RMR measurement?
4. Study 4: What is the status of RMR testing in the Australian high-performance sporting environment?

Study findings are outlined below. While they provide insight into factors that contribute to variability or errors in RMR measurements, they also highlight that further research is needed.

9.1 Novel findings

9.1.1 Study 1

There has been an increased acknowledgement of the underrepresentation of women in exercise science research (Cowley et al., 2021; Kuikman, McKay, et al., 2023; Kuikman, Smith, et al., 2023; Smith et al., 2022). While it is important to address this gender gap in research, female specific research must adopt Best Practice Guidelines for control of ovarian hormones (Elliott-Sale et al., 2021). Much of the previous research assessing changes in RMR across the MC or with HC usage (Benton et al., 2020) has not implemented these recommendations nor has this been assessed specifically in athletic cohorts. As such, it was unknown if changes in sex hormones across MC phase or with HC usage introduced a biological source of variability in RMR measurements. We assessed this while also implementing Best Practice Guidelines for control of ovarian hormones (Elliott-Sale et al., 2021) in a group of female athletes from the National Rugby League's Indigenous Women's Academy during a 5-week training camp. Attempts were made to assess changes across Phase 1, Phase 2 and Phase 4 of the MC. However, as we were only able to capture Phase 2 of the MC in one athlete, Phase 2 metrics were not included in our phase-based analysis. Additionally, menstrual disturbances were identified in 5 NC athletes during the training camp (n= 3 oligomenorrheic, n= 1 anovulatory, n= 1 luteal phase defect). This finding aligns with previous research reporting a greater prevalence of menstrual irregularities in athletes compared to sedentary populations (De Souza et al., 1998). This highlights the need to follow Best Practice Guidelines for control of ovarian hormones when assessing for changes across MC phase in athletes, so that subtle menstrual disturbances are identified, and measurements are occurring in the correct hormonal profile.

Contrary to the findings of others (Benton et al., 2020; Löfberg et al., 2024), we found no difference in RMR between Phase 1 (32.5±2.5 kcal·kg FFM^{-1}·day^{-1}) and Phase 4 (32.7±2.7 kcal·kg FFM^{-1}·day^{-1}) of the MC. Additionally, we failed to find an association between RMR and concentrations of estradiol (r=0.31, p=0.17), progesterone (r=0.06, p=0.79), or their ratio (r=0.11, p=0.67). Together, this provides evidence that variations in estrogen and progesterone across the MC do not contribute to biological variability in RMR measurements. The contrast between these findings and previous reports (Benton et al., 2020; Löfberg et al., 2024) can be attributed to several factors, including the failure of other studies to follow Best Practice Guidelines for control of ovarian hormones (Elliott-Sale et al., 2021). For instance, within a meta-analysis looking at changes in RMR across the MC, only ten of thirty studies verified MC phase (Benton et al., 2020). As such, measurements could have unknowingly occurred in the wrong phase of the MC. Another source of conflict could be the athletic calibre of study participants; our research included Tier 3 female athletes (McKay et al., 2022) with measurements occurring over a training camp, while, to our knowledge, no other study has investigated changes in RMR across the MC specifically in female athlete cohorts. As such, it is possible that physiological adaptations that occur with training overshadowed any changes in RMR due to variations in sex hormones across the MC, especially as previous reports of changes in RMR across the MC are small (~45 kcal·day^{-1}) (Benton et al., 2020).

We were unable to assess changes over Phase 2 of the MC. This phase of the MC was of interest as estrogen concentrations are highest at this time while progesterone concentrations remain low (Elliott-Sale et al., 2021). However, this phase lasts only 12-26 hours and requires resources to determine its presence that are unlikely to be available in an athlete's daily training environment (Elliott-Sale et al., 2021). Any changes in RMR that occur during Phase 2 of the MC are unlikely to have clinically meaningful implications for an athlete's energy needs. In

practical terms, by the time it is identified that an athlete is in phase 2 of the MC, hormonal variations and any potential effects on metabolic rate will have passed. However, if they exist as an example of biological variability, potential changes in RMR during this phase could have implications for research or clinical practice if it is being measured to screen for LEA. Practically though, it would be difficult for most institutions/organisations working with athletes to implement the procedures required to determine the hormonal environment of each female athlete *prior* to testing. As such, it is more realistic for organisations/institutions to gather self-reported data on an athlete's MC on the day of testing (i.e. length of MC and day of last bleed) and use this information to help interpret findings.

In addition to failing to detect any effect of MC phase on RMR, our study also found no differences between the RMR of NC athletes (32.6±2.5 kcal·kg FFM^{-1}·day^{-1}) and HC users (31.5±2.6 kcal·kg FFM^{-1}·day^{-1}). This agrees with previous research that has examined differences between NC athletes and COC users (Duhita et al., 2017, 2019; Eck et al., 1997; Jensen & Levine, 1998) as well as research looking at DMPA injection (Pelkman et al., 2001; Steward et al., 2016). We acknowledge the lack of homogeneity within the type of HC used by athletes in our study, with four athletes using the COC pill (but all different COC brands), seven athletes using a subdermal progestin implant and one athlete using DMPA injection. Additionally, we did not assess if there were differences in RMR between active pill and non-active pill taking days for COC users. Yet, as the large variations in sex hormones across the MC of NC athletes (6-fold increase in estrogen and 31-fold increase in progesterone) did not result in any variation in RMR measurements, it seems unlikely that there would be differences in RMR between active and inactive pill taking days as endogenous sex hormones remain unchanged (Elliott et al., 2005; Löfberg et al., 2024).

In conclusion, the findings of our study suggest that sex hormones do not make a detectable contribution to biological variability in RMR measurements in athletic cohorts. This suggests that MC phase and HC usage do not need to be standardised when measuring RMR in female athletes. However, noting that this is the first study to examine changes in RMR across MC phase and with HC usage specifically in female athletes, while also implementing Best Practice Guidelines for control of ovarian hormones, further research is needed with cohorts of athletes from different sports.

9.1.2 Study 2

Athletes often periodise ~2-4 weeks periods at an altitude of ~1600-2400 m within their training cycle (Stellingwerff et al., 2019). However, only two previous studies have looked at changes in RMR with low to moderate altitude exposure in athlete cohorts (Woods, Garvican-Lewis, et al., 2017; Woods, Sharma, et al., 2017). One of these studies reported a ~19% increase in the RMR of male and female middle-distance runners at the end of a 4-week altitude training camp at ~2200 m (Woods, Sharma, et al., 2017). The other study did not measure RMR at altitude but observed a trend for a ~5% decrease in the RMR of male and female rowers on return from a 12-day training camp at ~1800 m compared to pre-altitude RMR (Woods, Garvican-Lewis, et al., 2017). These studies provided preliminary support that altitude exposure may contribute to variability in RMR measurements, but were limited by their small sample size, and failure to control EA during the training camp (Woods, Garvican-Lewis, et al., 2017; Woods, Sharma, et al., 2017). As such, we sought to build on this research by (1) controlling EA throughout the duration of altitude exposure; and (2) investigating for a time course change in RMR by measuring RMR at 3 points during altitude exposure (36 hours exposure, 2 weeks exposure, 3 weeks exposure) as well as prior to and following altitude exposure while at sea level. Given that many athletes may be in a state of LEA during altitude training, and that the previously reported decrease in RMR post-altitude was thought to be due

to LEA (Woods, Garvican-Lewis, et al., 2017), we implemented a 7-day LEA intervention during the final week at altitude. This allowed us to investigate if changes in RMR with altitude exposure are moderated by LEA.

Similar to findings at high altitude (Hannon & Sudman, 1973; Mawson et al., 2000), we found a temporal effect of altitude exposure on RMR; compared to pre-altitude, RMR increased after 36 hours of altitude exposure (+5.3±3.1%), and two weeks altitude exposure (+4.9±4.9%), but was no longer elevated after three weeks altitude exposure (+1.7±4.2%). This suggests increases in RMR with low altitude exposure are temporal and disappear with more prolonged exposure. This has implications for RMR measurements undertaken at institutions/organisations located at a low to moderate altitude (i.e. Denver, Johannesburg, Mexico City), as athletes unaccustomed to this altitude may present with an elevated RMR. The ~5% increase in RMR that we observed was much less than the ~19% increase previously observed at the end of a 4-week altitude training camp at 2200 m (Woods, Sharma, et al., 2017). The smaller increase in RMR in our study may be due to the lower elevation of our training camp site (1800 m). Additional explanations include sex-based differences as our study included a female only cohort, whereas the previous study included a small cohort of both male (n=3) and female (n=2) athletes (Woods, Sharma, et al., 2017). Interestingly, the observed ~19% increase in RMR is also greater than what has been observed at even higher altitudes. For instance, measurements undertaken at an altitude of 4300 m found a ~17% increase in RMR on day 10 in men (Butterfield et al., 1992), while in women, a ~7% increase in RMR on day 3 but a return to sea-level values by day 6 was observed (Mawson et al., 2000). Evidently, more research is needed to determine if sex-based differences exist for changes in RMR with altitude exposure.

From pre-altitude to post-altitude, there was an unexpected downward trend in RMR (-3.9±7.2%; or -1.6 kcal·kg FFM^{-1}·day^{-1}). A similar decrease in pre-altitude to post-altitude RMR (-1.5 kcal·kg FFM^{-1}·day^{-1}) has been reported following 12 days at 1800 m, which was attributed to inadvertent LEA during the altitude training camp (Woods, Garvican-Lewis, et al., 2017). However, as we provided athletes in the current study with a controlled high EA diet throughout the training camp, the decrease in RMR from pre-altitude to post-altitude is unlikely to be due to LEA. Rather, this may have been the result of physiological adaptations that occur with altitude exposure, and/or increases in training load that occurred during the camp. For instance, an improved mitochondrial efficiency with altitude training (Gore et al., 2007; Murray & Horscroft, 2016) could contribute to a reduced RMR given that mitochondrial parameters have been linked to RMR in humans (Larsen et al., 2011). Future research is needed to assess if changes in RMR occur due to physiological adaptations with altitude exposure or training. Until this is determined, caution should be taken when measuring the RMR of an athlete immediately post-altitude exposure.

In addition to assessing for a time course change in RMR with altitude exposure, we implemented a LEA intervention during the final week of altitude and found that 7 days of LEA exposure while at altitude had no effect on RMR. Although it is possible that the LEA exposure was not of sufficient duration or magnitude to induce changes in RMR, other studies have reported decreases in RMR in female cohorts following 10 days of 25 kcal·kg FFM^{-1}·day^{-1} (Oxfeldt et al., 2024) and 3 days of 15 kcal·kg FFM^{-1}·day^{-1} (Hutson et al., 2024). Indeed, only one other study has failed to show a reduction in RMR with LEA in female athletes (Caldwell et al., 2024), although previous LEA interventions in men have not resulted in a reduction in RMR (Jurov et al., 2022; Kojima et al., 2020; Sim et al., 2024). An alternative explanation is that altitude exposure moderates the effect of LEA exposure on RMR and prevented a decrease

in RMR. For example, reductions in sympathetic nervous system activity are thought to contribute to reductions in RMR with LEA (Müller & Bosy-Westphal, 2013). Yet, altitude exposure is thought to increase sympathetic nervous system activity (Sander, 2016) and contribute to increases in RMR seen with altitude exposure (Moore et al., 1987). Evidently, this represents another area that requires further investigation in order to determine if altitude exposure moderates the effects of LEA on RMR.

Collectively, the results of study 2 highlight that low altitude exposure may introduce variability in RMR measurements to athletes both when exposed to altitude, and in the post-altitude period. It is important to note that the increase in RMR that we observed was small (50-75 kcal·day^{-1}) and is unlikely to have clinically significant implications for an athlete's total daily energy requirements. Finally, we did not find evidence that 7 days of LEA exposure alters the response of altitude on RMR.

9.1.3 Study 3

RMR measurements occur in an overnight fasted state with no exercise on the morning of testing. The requirement that RMR measurements occur in a fasted state is to avoid an artificially inflated RMR measurement due to TEF (Compher et al., 2006; Fullmer et al., 2015). However, this guideline only considers the time between the last food intake and a RMR measurement and not cumulative TEF over a 24-hour period. This is particularly relevant in situations of high EI, as TEF is proportional to EI (Quatela et al., 2016). A study published in 1980 reported that 24 hours of overfeeding resulted in a ~12% increase in BMR that was measured in a 14-15 hour fasted state (Dauncey, 1980). To our knowledge, no study has assessed the need to standardise EI the day prior to a RMR measurement. As such, it was unknown if differences in EI over 24 hours created artifacts in RMR measurements that occurred in an overnight fasted state. In addition to prior EI creating artifacts in RMR

measurement, prior exercise also needs to be considered. If there is an insufficient period of time between a bout of exercise and a RMR measurement, then RMR measurements may be falsely elevated due to EPOC. Yet, there are no clear guidelines as to how long exercise should be restricted prior to a RMR measurement (Fullmer et al., 2015). While many studies report restricting exercise for >24 hours prior to a RMR measurement (Sterringer & Larson, 2022), this may not be a realistic option for an athlete with high training loads. This study assessed if acutely manipulating diet and exercise for 24 hours would create artifacts in RMR measurements that occurred in an overnight fasted and rested state as per current recommendations (Compher et al., 2006; Fullmer et al., 2015).

Using a counterbalanced Latin square design, male and female endurance athletes (10M/10F) underwent 5 conditions that manipulated EI and EEE. The average EI on the energy restricted diet was 823 kcal·day^{-1} (range: 530-1275 kcal·day^{-1}) whereas the average EI on the energy surplus diet was 4128 kcal·day^{-1} (range: 2659-6479 kcal·day^{-1}). This resulted in an estimated TEF of 94 kcal·day^{-1} for the energy restricted diet and 463 kcal·day^{-1} for the energy surplus diet. Despite these extreme differences in EI and TEF, there was no effect of diet on RMR measurements that occurred the next morning in a 10-hour fasted state. Likewise, exercise had no effect on RMR measurements that occurred 12 hours post-exercise with participants either remaining sedentary or completing two bouts of cycling that resulted in an average cumulative cycling duration of ~210 minutes and EEE of ~1648 kcal·day^{-1}. These results suggest that exercise and diet do not need to be standardised the day prior to a RMR measurement.

To our knowledge, no other study has assessed the need to control EI the day prior to a RMR measurement. As mentioned, one previous study provided evidence that increased EI may artificially inflate morning RMR measurements (Dauncey, 1980). Contrary findings may be

due to differing methods, with this study measuring BMR with whole body indirect and direct calorimetry (Dauncey, 1980). The lack of change in RMR with exercise was surprising given past research demonstrating elevations in RMR with cycling of similar intensity but shorter duration (Francois et al., 2017; Hunter et al., 2017; Maehlum et al., 1986). However, this may relate to the different calibre of athletes as two of these studies involved untrained participants (Francois et al., 2017; Hunter et al., 2017). Our study was novel given the exercise that participants completed a workload similar to that of a high-level athlete in a real-life setting with both a morning and evening bout of exercise. There are some limitations as different results may by seen with other modes of exercise, such as running (Cunha et al., 2016), HIIT (Francois et al., 2017; Greer et al., 2015; Hunter et al., 2017), resistance exercise (Burt et al., 2014; Dolezal et al., 2000; Gillette et al., 1994; Greer et al., 2015; Hackney et al., 2008; Melby et al., 1993; Osterberg & Melby, 2000; Paoli et al., 2012; Paschalis et al., 2010; Schuenke et al., 2002) or following competition/matches (Carter et al., 2023; Hudson et al., 2020).

In conclusion, our results provide evidence that EI does not need to be standardised, and athletes can engage in their accustomed exercise routines on the day prior to a RMR measurement. However, HIIT and resistance exercise should be restricted the day prior to a RMR measurement, and RMR measurements should not be scheduled the day after a competition or match until it is determined if this introduces error into RMR measurements.

9.1.4 Studies 1 & 3

Alongside RMR measurements, DXA scans were used to estimate body composition as this allowed us to calculate relative RMR. However, this also allowed us to assess the need to standardise MC phase and HC usage (study 1), and diet and exercise (study 3) when using a DXA scan to estimate body composition. In study 1, we found that MC phase and HC usage

had no effect on DXA derived body composition estimates. This suggests that MC phase and HC usage do not need to be standardised when using DXA to estimate body composition. While not a consistent finding (Thompson et al., 2021), this is in agreement with others reporting no difference in DXA estimates of body composition between the mid-follicular and luteal phase of the MC (Jürimäe et al., 2011; Koşar et al., 2022; Ong et al., 2022b). In study 3, we found that DXA derived estimates of FFM and LBM were greater following conditions of high EI both with and without exercise compared to LEA diets. Previous studies have reported that error is introduced into DXA derived body composition estimates when scans occurred following food and/or fluid consumption (rather than in a fasted state) (Nana et al., 2012; Ong et al., 2022a; Tinsley et al., 2017), with dehydration (Going et al., 1993; Rodriguez-Sanchez & Galloway, 2015; Toomey et al., 2017), and with increased glycogen stores (Bone et al., 2017; Burke et al., 2011; Rouillier et al., 2015). We believe that the difference in FFM and LBM we observed was due to differences in the volume of food provided across conditions. If this artifact was created by difference in glycogen stores, then we would have expected to see differences in limb estimates of FFM and LBM (Bone et al., 2017; Rouillier et al., 2015). Yet, we found no difference in body composition estimates of limbs across conditions, but rather just differences in estimates of trunk. Reductions in DXA derived estimates of FFM and LBM have been reported with dehydration (Going et al., 1993; Rodriguez-Sanchez & Galloway, 2015; Toomey et al., 2017), and participants in our study were more dehydrated following the high energy diet with exercise compared to the LEA diet with rest. If the observed difference was due to differences in hydration across conditions, then we would have expected to see a reduced FFM and LBM with the high energy diet with exercise, rather than the observed increased. As such, it seems unlikely that differences in DXA derived estimates of body composition were due to differences in hydration across conditions. Regardless of the

underlying cause, our study provides novel evidence that diet should be standardised in the 24 hours that precedes a DXA scan that is being used to estimate body composition.

9.1.5 Study 4

The final study of this PhD examined the use of RMR measurements within the Australian high-performance sport environment. This was achieved by interviewing practitioners across the Australian High Performance Sport System who measure RMR in high performance athletes. This information was used to qualitatively assess barriers and enablers to measuring RMR in the high-performance environment. Information from these interviews was also used to create an insight report for the Australian Institute of Sport (see Appendix 11.2) and update Best Practice Guideline for RMR measurements (see Appendix 11.1).

Although the interviewed practitioner reported the use of RMR measurements to assess EA status rather than for dietary planning purposes, most expressed scepticism about its use as an LEA indicator. This scepticism was mostly related to the threshold used to indicate LEA and potential errors in the measurements due to athlete presentation, testing equipment, and/or the environment of RMR testing. Many practitioners also felt that they were not properly trained to measure RMR and described confusion over whether the responsibility for measurements should rest with dietitians or physiologists. Despite this, RMR measurements were considered a piece of the LEA assessment puzzle and useful when measured longitudinally to monitor changes in RMR over time.

These interviews were in part conducted to gain insight on contextual factors that must be considered when developing Best Practice Guidelines for RMR measurements in athletic cohorts. Interviews also identified areas of high research priority, as well as areas of discrepancies in practices across institutions and organisations that may contribute to

variability in RMR measurements. Updated Best Practice Guidelines for measuring RMR in the Australian High Performance Sport System when using a metabolic cart can be found in Appendix 11.1, and the insight report can be found in Appendix 11.2.

9.2 Reflection on RMR measurements as an indicator of LEA

Prior to commencing my PhD, I conducted a meta-analysis looking at changes in indicators of LEA in overreached athletes (Kuikman et al., 2022). Notably, this meta-analysis was completed before the publication of the REDs CAT2 (Stellingwerff et al., 2023), so there was no validation of LEA indicators or classification of these indicators as primary, secondary or potential. Of all the LEA indicators we analysed, I thought RMR measurements had the most evidence to support their use as an indicator of LEA. However, I failed to realise the difficulty in precisely measuring RMR and lack of knowledge regarding what constitutes a "normal" RMR. As I dug into the RMR research for my PhD and began measuring RMR myself, I started to become more sceptical of RMR measurements. With the end of my thesis in sight, this scepticism in RMR measurements was further confirmed when I received news of a systematic error that had occurred in the calculation of RMR when using the Douglas bag method. This error had impacted the RMR measurements undertaken for Study 1 and Study 3 due to the incorrect calculation of minute ventilation, resulting in RMR measurements being systematically elevated for both studies. When using the Douglas bag method, there is a small amount of gas that is lost from the Douglas bag during the sampling process. The volume of gas lost needs to be accounted for when later determining the total volume of air in the Douglas bag, so that minute ventilation can then be calculated. The gas volume lost during sampling had simply been added to the uncorrected minute ventilation rather than being added to the numerator with the sum then being divided by time:

Incorrect equation Correct equation

Minute ventilation = (Volume ÷ Time) + *Volume lost* = (Volume + *Volume lost*) ÷ Time

It brought me back to high school algebra and learning the orders of operation through the BEDMAS rule. This small error had a knock-on effect, leading to an incorrect value of VO_2 and VCO_2 being introduced into the Weir equation to estimate energy expenditure. For study 1, RMR measurements were elevated by 60±7 kcal·day^{-1} (~3.7%), and for study 3, RMR measurements were elevated by 58±7 kcal·day^{-1} (~3.4%). Fortunately, none of the major results of either study changed as there was a strong linear correlation between the incorrect and correct RMR measurement for both study 1 (r=0.999, 95% CI: [0.998, 1.000]) and study 3 (r=0.999, 95% CI: [0.999, 1.000]). Because study 1 had already been published, an erratum for the publication was prepared (see chapter 5). Thankfully, the error was identified prior to publication of study 3 and data were corrected at the proofing stage.

While the primary scientific outcomes of these studies did not change, the data correction altered the number of athletes who presented with RMR values that were considered suppressed, using RMR ratio and relative RMR thresholds. In study 1, 5 participants were newly classified as having suppressed RMR measurements, while in study 3, 4 additional athletes joined this group. These findings represent a false negative outcome in the original findings for 9 of 43 athletes (~21%). While this may be perceived as a major error or change in interpretation, the magnitude of error in RMR was small (<4%) and within the 3-5% day-to-day variation in RMR (Compher et al., 2006). This highlights that small errors or variations in RMR measurement can create a big impact on the interpretation of the outcome. However, it does not mean that these 9 athletes (or the other 18 athletes) were experiencing a true reduction in RMR indicating metabolic suppression. Indeed, these thresholds need to be interpreted with caution and it is notable that all the new incidences of a "suppressed" RMR measurement were derived from the adjusted values falling <30 kcal·kg FFM^{-1}·day^{-1}. Yet, the origin of this

threshold is questionable, and it is most widely attributed to a paper which re-analysed sleeping metabolic rate data (Westerterp, 2003) as a function of FFM, with the dashed line through the data and origin having a slope of 30 kcal·kg FFM^{-1}·day^{-1} (Loucks et al., 2011). Table 9.1 highlights sources of variability within the original study from which the SMR data was obtained (Westerterp, 2003) as well as the study that validated the RMR ratio thresholds (Strock et al., 2020) in comparison to the studies that make up this thesis. The notable differences, such as differences in athletic tier, age, sex, or quantity of FFM, may mean that these thresholds cannot be generalised to our study participants. Different methods used to measure metabolic rate may introduce an even greater source variability. For instance, a ventilated hood may result in lower RMR measurements than a mouthpiece and nose clip (Forse, 1993; Roffey et al., 2006) and SMR is lower than RMR (Kumahara et al., 2004). Evidently, these thresholds may not be appropriate for the participants in our study or compatible with the methods that we used to measure RMR.

Table 9.1. Participant characteristics and procedures for measuring metabolic rate in studies 1-3 in comparison to the study that validated the RMR ratio thresholds (Strock et al., 2020) and the study that led to the use of a relative RMR threshold (Westerterp, 2003) to screen for low energy availability.

	RMR ratio (Strock et al., 2020)	Relative RMR (Westerterp, 2003)	Study 1	Study 2	Study 3
Biological sources of variability					
Training status	≥2 hrs/wk exercise	Unknown	Tier 3 Rugby League	Tier 3-5 Race walkers	Tier 2-3 Endurance athletes
Sex	Female	Male & Female	Female	Female	Male & Female
Age (yrs)	23	27	21.6±3.4	26.5±6.5	35.4±8.3
Height (cm)	165.3	174.6	165.1±4.50	165.7±5.34.7	172.9±9.8
Body mass (kg)	58.9	73.2	76.4±12.7	53.6±5.3	73.4±13.8
FFM (kg)	43.4	53.7	51.3±5.0	43.2±3.9	55.1±10.9
FM (kg)	15.3	18.8	25.2±9.5	10.4±2.9	18.3±6.7
Technical sources of variability					
Indirect calorimetry system	Metabolic cart	Whole body	Douglas bag	Metabolic cart	Douglas bag

Gas collection device	Hood	Whole body	Mouthpiece	Mouthpiece	Mouthpiece
Rest period (min)	30-45	SMR measured	10	10	10
Familiarisation length (min)	Not mentioned	N/A	15	10	15
Measurement length (min)	30	Not mentioned	2 x 10	25	2 x 10
Gas exchange selection	Steady state	Not mentioned	Mean of bags	Mean of min 2-23	Mean of bags

DXA, dual-energy X-ray absorptiometry; FFM, fat free mass; FM, fat mass; RMR, resting metabolic rate; SMR; sleeping metabolic rate; VO2, maximal aerobic capacity

In study 1, ~48% of athletes, and in study 3, ~80% of athletes presented with a suppressed RMR measurement for at least 1 measurement (see Table 9.2). However, this was mostly due to relative RMR falling below the threshold of <30 kcal·kg FFM^{-1}·day^{-1}. Using the RMR ratio thresholds alone, the number of athletes that presented with a suppressed RMR measurement decreased to ~17% of athletes in study 1, and ~50% in study 3. Interestingly, none of the athletes in study 2 had a RMR measurement that would be considered suppressed despite being at high risk of LEA due to the sport (race walking) being weight sensitive and having a high EEE (Torstveit et al., 2023). These athletes had smaller FFM (43.2±3.9 kg) than athletes in both study 1 (51.3±5.0 kg) and study 3 (55.1±10.9 kg), so they would naturally present with a higher relative RMR (Heymsfield et al., 2002; Wang et al., 2000) and thus, be less likely to present with a relative RMR <30 kcal·kg FFM^{-1}·day^{-1}. An alternative explanation for the outlying results of Study 2 lies with methodological differences, as this study was the only one to use a metabolic cart rather than the Douglas bag method.

Table 9.2. Number of athletes that presented with a suppressed RMR measurement using relative RMR or a RMR ratio for at least 1 testing visit.

	Study 1 (n=23F)	Study 2 (n=19F)	Study 3 (n=10M/10F)
Suppressed RMR using any method	11F	0	8M/8F
Relative RMR <30 kcal·kgFFM^{-1}·day^{-1}	11F	0	8M/8F
HB RMR ratio <0.90	4F	0	4M/2F
Cunningham 1980 RMR ratio <0.90	2F	0	4M/4F
Cunningham 1991 RMR ratio <0.92	0	0	3M/2F
DXA predicted RMR ratio <0.94	N/A	N/A	4M/5F

DXA, Dual-energy X-ray absorptiometry; F, female; HB, Harris Benedict; M, male; RMR, resting metabolic rate.

Despite the lack of evidence to support its use, RMR measurements are widely used within the literature as an indicator of LEA in athletic cohorts. Indeed, I was able to locate 45 articles that have used RMR measurements as an indicator of LEA with the first publication in 2007 within the context of the female athlete triad. Of these 45 articles, 36 (80%) have been published since 2018. This increased interest in RMR as an indicator of LEA may align with the introduction of REDs in 2014 with the consensus statement stating that "a measurement of RMR via indirect calorimetry may provide confirmation of a suppressed metabolism secondary to low EA (Mountjoy et al., 2014)." Nearly 10 years later, and the 2023 updated consensus statement on REDs no longer supports the use of RMR measurements as a primary or secondar indicator of LEA (Mountjoy et al., 2023). The lack of support is not because the use of RMR measurements as an indicator of LEA has been disproven, but rather that more research is needed to support

its use. Given this, I have not given up on the potential use of RMR measurements as an indicator of LEA, but further research is needed.

9.3 Reflection on conducting research in female athletes

To address the sex-based gaps in research, all studies included in this body of work included female-only cohorts or, in the case of one study, included men to allow sex-based comparisons. Prior to commencing my studies, I thought that it would be more difficult to control the diets of female athletes than their male counterparts. In reality, I found that difficulties in diet planning seemed to relate more to the characteristics of the sport than the sex of the athlete. For instance, there were few dietary restrictions or special dietary needs among the athletes in study 1 (rugby league players). On the other hand, the athletes in study 2 (race walkers) had extensive dietary requirements with all but 3 athletes having special dietary requests that needed to be considered when planning diets. Study 3 was the only study to include both male and female athletes, but there was no notable difference in diet planning between the sexes in this study.

While there were no notable differences in diet planning between genders, the requirement to control for or characterise menstrual status was of note. Controlling for MC phase, as was needed in study 1, proved to be a time-consuming process that required extensive resources. I cannot take credit for tracking and ensuring that athletes were in the correct phase of the MC on the day of testing; this was a true team effort that required extensive expertise, planning, and resources. The results of this study were a great reminder that the "perfect" 28-day cycle is uncommon, whereas subtle menstrual irregularities are frequent. This study also made me re-think the relevance of assessing for changes in RMR during Phase 2 and Phase 3 of the MC. Even if changes in RMR during these unique hormonal profiles had been captured, they appear academic rather than pragmatic since I'm not confident these findings would change

interpretations of EA status or the nutrition recommendations given to athletes. Study 2 required significant effort and resource to characterise the menstrual status of each participant, including having participants track MC prior to commencing and throughout the study, measuring sex hormones periodically throughout the study, and having athletes test for ovulation. Some of the athletes in this study had poor compliance with monitoring ovulation and tracking their MC daily, which may relate to the burden of these tasks and/or language barriers. I spent a great deal of time analysing this data and trying to characterise the menstrual status of participants, but this did not change any of the results or conclusion of our study. However, it was still important to collect this information as the planning of this study preceded the findings of study 1, which found no effect of MC phase or HC usage on RMR. For study 3, we only include participants that were COC users. This inclusion criteria narrowed down the pool that could be recruited and it required a lot of extra effort to ensure a homogenous menstrual status among the female participants. This is something to consider for studies recruiting participants of a homogenous menstrual status. There is no doubt that the lack of women in research needs to be addressed, and this research does need to be of high quality. While ensuring that all studies in this thesis included women required extra effort and resources, it was vital to ensuring that we were not perpetuating gender gaps in research. This has also allowed me to develop valuable skills and knowledge surrounding methodological considerations for the control of ovarian hormones that will be useful in future research endeavours.

9.4 Reflection on doing a PhD

Within weeks of landing in Australia, I was thrown into helping with a research-embedded training camp study. I was so excited to learn from the best. I was amazed by the level of dietary and exercise control (they were weighing out every meal!) that was being implemented and knew that I was exactly where I needed to be to gain extensive experience in sports nutrition

research. Fast forward three years, and the knowledge and skill set that I have acquired during this PhD have far exceeded my expectations. I have gained extensive experience in planning and implementing dietary interventions for research studies. This includes planning diets based on athlete needs and preferences, and adjusting this plan when needed. The skill set that I have attained extends beyond implementing nutrition interventions to also measuring RMR and body composition via DXA scans, both of which are valuable skills to have as a dietitian. Beyond skills that I have obtained that are of value to a career in sports nutrition, I have obtained skills that are transferrable to any future career. This includes the ability to problem solve and pivot from the original plan, answer questions by delving into research, effectively manage time, collaborate with others, and so much more. My knowledge has not only greatly expanded in sports nutrition, but also exercise physiology. I find this particularly valuable as my training in dietetics did not offer any formal education in exercise physiology, and I feel that this knowledge is often missing for dietitians working in sport. As I've gone through this PhD, I've come to realise that there are more questions than answers. Answering these questions doesn't happen quickly, and it often comes with even more questions that need to be answered. Working in research requires delayed gratification, but it is an extremely rewarding experience.

9.5 Future direction

While this thesis has addressed several notable gaps in the literature, several areas require further investigation, which are outlined in detail below. This is certainly not an exhaustive list of questions, but rather, high priority questions that must be addressed before RMR measurements will be useful within the context of LEA screening.

1) What is the best way to measure RMR? A uniform approach is needed when measuring RMR both across the high-performance sport system and within research. As such, clear standards

and guidelines are needed when measuring RMR in athletic cohorts with previous recommendations not being specific to athletes (Compher et al., 2006; Fullmer et al., 2015). Yet, there are contextual factors specific to the athlete and their environment that must be considered. However, before this can be determined, there are some research questions that need to be addressed. Questions that arose through my own experience measuring RMR as well as when interviewing practitioners during study 4 included: Does athlete discomfort or anxiety during the measurement introduce error, such as discomfort associated with the mouthpiece and nose clip, or lying on an uncomfortable bed during the measurement? What is the most suitable length of familiarisation with the mouthpiece prior to commencing the measurement? Does unexpected noise in the surrounding area create artefacts in RMR measurements? Can listening to music/white noise increase measurement accuracy in athletes unable to stay awake or having difficulties laying still for a prolonged period? Answering these questions will allow for the determination of best practice guidelines for RMR measurements that can be followed uniformly so that variability is reduced, and error is eliminated.

2) Does an athlete's training load impact RMR? It must be determined if an athlete's training load independent of LEA contributes to variation in RMR measurements. In non-athletic populations, this has been described as a "compensation model" by which increases in level of activity are accompanied by a reduction in RMR to keep total energy expenditure within a constrained energy budget (Careau et al., 2021). Previous findings of a decreased RMR following intensified training periods or overtraining are thought to be due to LEA (Kuikman et al., 2022; Stellingwerff et al., 2021). This seems logical as these studies have not controlled for EI and the increased EEE is not always matched by increased EI (Drenowatz et al., 2012; Stubbs et al., 2004). However, it's also possible that physiological adaptations that occur with intensified training are contributing to the decrease in RMR. Study 2 provided preliminary

evidence of this as the decrease in RMR that occurred post-altitude training camp was unlikely to have been caused by LEA, due to the implementation of an EA-controlled diet/exercise protocol. It's possible that this was the result of physiological adaptations that occurred with altitude exposure and/or increases in training load throughout the training camp. RMR measurements cannot be used as an indicator of LEA until it is determined if variations in training load are contributing to variability in RMR measurements independent of LEA.

3) What is a "normal" RMR? Another notable gap in the literature is the relative RMR threshold used to indicate adaptation to LEA. By building a normative database for RMR, that adjusts for factors that may impact the RMR to FFM relationship (i.e. athlete physique, race, age, sex), there could be an increased understanding of the range of an athlete's "normal" RMR. This could also provide insight into novel ways of assessing an athlete's RMR, such as indexing RMR to regional estimates of FFM. For instance, as most metabolically active tissue is within the trunk, indexing RMR to trunk FFM rather than total FFM may provide greater insight into the slowing of metabolically active tissue due to LEA. Until there is a greater understanding of what constitutes a "normal" RMR, RMR measurements should only occur longitudinally so that changes in an athlete's RMR are being tracked overtime.

4) *Does resistance exercise or high intensity exercise need to be restricted prior to a RMR measurement?* While we found no effect of exercise on next day RMR measurements in study 3, these findings are specific to exercise in the form of moderate intensity cycling. Of course, athletes engage in many other forms of exercise, and it is possible that EPOC from this exercise could carry over and create artefacts in next-day RMR measurements. Further research is needed to assess if high intensity exercise (i.e. interval sessions) and resistance exercise needs to be restricted the day prior to a RMR measurement.

9.6 Conclusion

This series of research studies investigated sources of variability and error in RMR measurements and addressed several major gaps in the literature. The key findings can be summarised as followed: 1) MC phase does not contribute to variability in RMR measurements in female athletes 2) HC usage does not contribute to variability in RMR measurements in female athletes 3) RMR is acutely increased with low altitude exposure but returns to baseline values with more prolonged exposure in female athletes 4) 7 days of LEA while at altitude does not impact RMR in female athletes 5) Large variations in EI does not introduce error into next day RMR measurements 6) Prolonged continuous cycling at moderate intensity does not introduce error into next day RMR measurements 7) RMR measurements are perceived to be a piece of the puzzle when assessing for LEA and valuable when measurements occur longitudinally and 8) there are still multiple barriers that need to be addressed when measuring RMR in the high-performance sport environment. A uniform approach is needed when measuring RMR, and there is a need for further research assessing sources of variability and error before RMR measurements can be used as an indicator of LEA.

References

Abboud, G. J., Greer, B. K., Campbell, S. C., & Panton, L. B. (2013). Effects of load-volume on EPOC after acute bouts of resistance training in resistance-trained men. *The Journal of Strength and Conditioning Research*, *27*(7), 1936–1941.

Abernethy, D. R., & Todd, E. L. (1985). Impairment of caffeine clearance by chronic use of low-dose oestrogen-containing oral contraceptives. *European Journal of Clinical Pharmacology*, *28*(4), 425–428.

Acheson, K. J., Zahorska-Markiewicz, B., Pittet, P., Anantharaman, K., & Jéquier, E. (1980). Caffeine and coffee: Their influence on metabolic rate and substrate utilization in normal weight and obese individuals. *American Journal of Clinical Nutrition*, *33*(5), 989–997.

Ackerman, K. E., Rogers, M. A., Heikura, I. A., Burke, L. M., Stellingwerff, T., Hackney, A. C., Verhagen, E., Schley, S., Saville, G. H., Mountjoy, M., & Holtzman, B. (2023). Methodology for studying Relative Energy Deficiency in Sport (REDs): a narrative review by a subgroup of the International Olympic Committee (IOC) consensus on REDs. *British Journal of Sports Medicine*, *57*(17), 1136–1147.

Acosta, F. M., Sanchez-Delgado, G., Martinez-Tellez, B., Alcantara, J. M. A., Llamas-Elvira, J. M., & Ruiz, J. R. (2021). Diurnal variations of cold-induced thermogenesis in young, healthy adults: A randomized crossover trial. *Clinical Nutrition*, *40*(10), 5311–5321.

Adzika Nsatimba, P. A., Pathak, K., & Soares, M. J. (2016). Ethnic differences in resting metabolic rate, respiratory quotient and body temperature: a comparison of Africans and European Australians. *European Journal of Nutrition*, *55*(5), 1831–1838.

Alam, D. S., Hulshof, P. J. M., Roordink, D., Meltzer, M., Yunus, M., Salam, M. A., & van Raaij, J. M. A. (2005). Validity and reproducibility of resting metabolic rate measurements in rural Bangladeshi women: Comparison of measurements obtained by Medgem™ and by Deltatrac™ device. *European Journal of Clinical Nutrition*, *59*(5), 651–657.

Albu, J., Shur, M., Curi, M., Murphy, L., Heymsfield, S. B., & Pi-Sunyer, F. X. (1997). Resting metabolic rate in obese, premenopausal black women. *American Journal of Clinical Nutrition*, *66*, 531–539.

Alcantara, J. M. A., Galgani, J. E., Jurado-fasoli, L., Dote-montero, M., Merchan-Ramirez, E., Ravussin, E., Ruiz, J. R., & Sanchez-Delgado, G. (2022). Validity of four commercially available metabolic carts for assessing resting metabolic rate and respiratory exchange ratio in non-ventilated humans. *Clinical Nutrition*, *41*(3), 746–754.

Alcantara, J. M. A., Hausen, M., Itaborahy, A., & Freire, R. (2024). Impact of equation choice on resting metabolic rate in high-level men and women athletes. *Journal of the American Nutrition Association*, *43*(5), 421–429.

Alcantara, J. M. A., Jurado-Fasoli, L., Dote-Montero, M., Merchan-Ramirez, E., Amaro-Gahete, F. J., Labayen, I., Ruiz, J. R., & Sanchez-Delgado, G. (2023). Impact of methods for data selection on the day-to-day reproducibility of resting metabolic rate assessed with four different metabolic carts. *Nutrition, Metabolism and Cardiovascular Diseases*, *33*(11), 2179–2188.

Alcantara, J. M. A., Sanchez-Delgado, G., Martinez-Tellez, B., Merchan-Ramirez, E., Labayen, I., & Ruiz, J. R. (2018). Congruent validity and inter-day reliability of two breath by breath metabolic carts to measure resting metabolic rate in young adults. *Nutrition, Metabolism and Cardiovascular Diseases*, *28*(9), 929–936.

Alfonzo-González, G., Doucet, E., Bouchard, C., & Tremblay, A. (2006). Greater than predicted decrease in resting energy expenditure with age: Cross-sectional and longitudinal evidence. *European Journal of Clinical Nutrition*, *60*(1), 18–24.

Almuzaini, K. S., Potteiger, J. A., & Green, S. B. (1998). Effects of split exercise sessions on excess postexercise oxygen consumption and resting metabolic rate. *Canadian Journal of Applied Physiology*, *23*(5), 433–443.

Arciero, P. J., Bougopoulos, C. L., Nindl, B. C., & Benowitz, N. L. (2000). Influence of age on the thermic response to caffeine in women. *Metabolism: Clinical and Experimental*, *49*(1), 101–107.

Arciero, P. J., Gardner, A. W., Calles-Escandon, J., Benowitz, N. L., & Poehlman, E. T. (1995). Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *American Journal of Physiology - Endocrinology and Metabolism*, *268*(6), 192–198.

Arciero, P. J., Goran, M. I., & Poehlman, E. T. (1993). Resting metabolic rate is lower in women than in men. *Journal of Applied Physiology*, *75*(6), 2514–2520.

Arciero, P. J., Smith, D. L., & Calles-Escandon, J. (1998). Effects of short-term inactivity on glucose tolerance, energy expenditure, and blood flow in trained subjects. *Journal of Applied Physiology*, *84*(4), 1365–1373.

Astrup, A., Toubro, S., Cannon, S., Hein, P., Breum, L., & Madsen, J. (1990). Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. *American Journal of Clinical Nutrition*, *51*(5), 759–767.

Audrain, J. E., Klesges, R. C., DePue, K., & Klesges, L. M. (1991). The individual and combined effects of cigarette smoking and food on resting energy expenditure. *International Journal of Obesity*, *15*(12), 813–821.

Bakker, L. E. H., Boon, M. R., van der Linden, R. A. D., Arias-Bouda, L. P., van Klinken, J. B., Smit, F., Verberne, H. J., Jukema, J. W., Tamsma, J. T., Havekes, L. M., van Marken Lichtenbelt, W. D., Jazet, I. M., & Rensen, P. C. N. (2014). Brown adipose tissue volume in healthy lean south Asian adults compared with white Caucasians: a prospective, case-controlled observational study. *The Lancet Diabetes & Endocrinology*, *2*(3), 210–217.

Balogh, A., Henschel, L., Klinger, G., Vollanth, R., Börner, A., & Kuhnz, W. (1995). Influence of ethinylestradiol-containing combination oral contraceptives with gestodene or levonorgestrel on caffeine elimination. *European Journal of Clinical Pharmacology*, *48*(2), 161–166.

Bärtsch, P., & Saltin, B. (2008). General introduction to altitude adaptation and mountain sickness. *Scandinavian Journal of Medicine & Science in Sports*, *18*(Suppl 1), 1–10.

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.

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.

Bell, C., Seals, D. R., Monroe, M. B., Day, D. S., Shapiro, L. F., Johnson, D. G., & Jones, P. P. (2001). Tonic sympathetic support of metabolic rate is attenuated with age, sedentary lifestyle, and female sex in healthy adults. *Journal of Clinical Endocrinology and Metabolism*, *86*(9), 4440–4444.

Belza, A., Toubro, S., & Astrup, A. (2007). The effect of caffeine, green tea and tyrosine on thermogenesis and energy intake. *European Journal of Clinical Nutrition*, *63*(1), 57–64.

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.

Bielinski, R., Schutz, Y., & Jequier, E. (1985). Energy metabolism during the postexercise recovery in man. *American Journal of Clinical Nutrition*, *42*(1), 69–82.

Bittencourt, V. Z., Freire, R., Alcantara, J. M. A., Loureiro, L. L., de Oliveira, T. M., Cahuê, F. L. C., Itaborahy, A., & Pierucci, A. P. T. R. (2023). Effect of gas exchange data selection methods on resting metabolic rate estimation in young athletes. *PLoS ONE*, *18*(9).

Blond, E., Maitrepierre, C., Normand, S., Sothier, M., Roth, H., Goudable, J., & Laville, M. (2011). A new indirect calorimeter is accurate and reliable for measuring basal energy expenditure, thermic effect of food and substrate oxidation in obese and healthy subjects. *E-SPEN*, *6*(1), e7–e15.

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.

Bone, J. L., Ross, M. L., Tomcik, K. A., Jeacocke, N. A., Hopkins, W. G., & Burke, L. M. (2017). Manipulation of muscle creatine and glycogen changes dual x-ray absorptiometry estimates of body composition. *Medicine and Science in Sports and Exercise*, *49*(5), 1029–1035.

Borges, J. H., Guerra-Júnior, G., & Gonçalves, E. M. (2019). Methods for data analysis of resting energy expenditure measured using indirect calorimetry. *Nutrition*, *59*, 44–49.

Børsheim, E., & Bahr, R. (2003). Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. *Sports Medicine*, *33*(14), 1037–1060.

Bosy-Westphal, A., Eichhorn, C., Kutzner, D., Illner, K., Heller, M., & Müller, M. J. (2003). The age-related decline in resting energy expenditure in humans is due to the loss of fat-free mass and to alterations in its metabolically active components. *The Journal of Nutrition*, *133*(7), 2356–2362.

Bosy-Westphal, A., Hinrichs, S., Jauch-Chara, K., Hitze, B., Later, W., Wilms, B., Settler, U., Peters, A., Kiosz, D., & Müller, M. J. (2008). Influence of partial sleep deprivation on energy balance and insulin sensitivity in healthy women. *Obesity Facts*, *1*(5), 266–273.

Bowden, V. L., & McMurray, R. G. (2000). Effects of training status on the metabolic responses to high carbohydrate and high fat meals. *International Journal of Sport Nutrition and Exercise Metabolism*, *10*(1), 16–27.

Bracco, D., Ferrarra, J. M., Arnaud, M. J., Jequier, E., & Schutz, Y. (1995). Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women. *American Journal of Physiology - Endocrinology and Metabolism*, *269*(4 Pt 1), 671–678.

Broeder, C. E., Burrhus, K. A., Svanevik, L. S., & Wilmore, J. H. (1992). The effects of aerobic fitness on resting metabolic rate. *American Journal of Clinical Nutrition*, *55*(4), 795–801.

Brychta, R. J., & Chen, K. Y. (2017). Cold-induced thermogenesis in humans. *European Journal of Clinical Nutrition*, *71*(3), 345–352.

Buchholz, A. C., Rafii, M., & Pencharz, P. B. (2001). Is resting metabolic rate different between men and women? *British Journal of Nutrition*, *86*(6), 641–646.

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.

Burke, L. M., Ackerman, K. E., Heikura, I. A., Hackney, A. C., & Stellingwerff, T. (2023). Mapping the complexities of Relative Energy Deficiency in Sport (REDs): development of a physiological model by a subgroup of the International Olympic Committee (IOC) Consensus on REDs. *British Journal of Sports Medicine*, *57*(17), 1098–1108.

Burke, L. M., Hawley, J. A., Wong, S. H. S., & Jeukendrup, A. E. (2011). Carbohydrates for training and competition. *Journal of Sports Sciences*, *29*(1), S17-27.

Burke, L. M., Lundy, B., Fahrenholtz, I. L., & Melin, A. K. (2018). Pitfalls of conducting and interpreting estimates of energy availability in free-living athletes. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(4), 350–363.

Burt, D. G., Lamb, K., Nicholas, C., & Twist, C. (2014). Effects of exercise-induced muscle damage on resting metabolic rate, sub-maximal running and post-exercise oxygen consumption. *European Journal of Sport Science*, *14*(4), 337–344.

Butterfield, G. E., Gates, J., Fleming, S., Brooks, G. A., Sutton, J. R., & Reeves, J. T. (1992). Increased energy intake minimizes weight loss in men at high altitude. *Journal of Applied Physiology*, *72*(5), 1741–1748.

Buxton, O. M., Cain, S. W., O'Connor, S. P., Porter, J. H., Duffy, J. F., Wang, W., Czeisler, C. A., & Shea, S. A. (2012). Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Science Translational Medicine*, *4*(129).

Byrne, H. K., & Wilmore, J. H. (2001). The relationship of mode and intensity of training on resting metabolic rate in women. *International Journal of Sport Nutrition and Exercise Metabolism*, *11*(1), 1–14.

Calcagno, M., Kahleova, H., Alwarith, J., Burgess, N. N., Flores, R. A., Busta, M. L., & Barnard, N. D. (2019). The thermic effect of food: A review. *Journal of the American College of Nutrition*, *38*(6), 547–551.

Caldwell, H. G., Jeppesen, J. S., Lossius, L. O., Atti, J. P., Durrer, C. G., Oxfeldt, M., Melin, A. K., Hansen, M., Bangsbo, J., Gliemann, L., & Hellsten, Y. (2024). The whole-body and skeletal muscle metabolic response to 14 days of highly controlled low energy availability in endurance-trained females. *The FASEB Journal*, *38*(21).

Cane J, O'Connor D, Michie S. Validation of the theoretical domains framework for use in behaviour change and implementation research. *Implementation Science*. 2012;7(37), 7–37.

Cannon, B., & Nedergaard, J. (2011). Nonshivering thermogenesis and its adequate measurement in metabolic studies. *Journal of Experimental Biology*, *214*(2), 242–253.

Careau, V., Halsey, L. G., Pontzer, H., Ainslie, P. N., Andersen, L. F., Anderson, L. J., Arab, L., Baddou, I., Bedu-Addo, K., Blaak, E. E., Blanc, S., Bonomi, A. G., Bouten, C. V. C., Buchowski, M. S., Butte, N. F., Camps, S. G. J. A., Close, G. L., Cooper, J. A., Das, S. K., … Speakman, J. R. (2021). Energy compensation and adiposity in humans. *Current Biology*, *31*(20), 4659-4666.e2.

Carlsohn, A., Scharhag-Rosenberger, F., Cassel, M., & Mayer, F. (2011). Resting metabolic rate in elite rowers and canoeists: Difference between indirect calorimetry and prediction. *Annals of Nutrition and Metabolism*, *58*(3), 239–244.

Carlsson, E., Helgegren, H., & Slinde, F. (2005). Resting energy expenditure is not influenced by classical music. *Journal of Negative Results in BioMedicine*, *4*,6.

Carpenter, W. H., Fonong, T., Toth, M. J., Ades, P. A., Calles-Escandon, J., Walston, J. D., & Poehlman, E. T. (1998). Total daily energy expenditure in free-living older African-Americans and Caucasians. *American Journal of Physiology - Endocrinology and Metabolism*, *274*(1), 96–101.

Carter, J. L., Lee, D. J., Perrin, C. G., Ranchordas, M. K., & Cole, M. (2023). Significant changes in resting metabolic rate over a competitive match week are accompanied by an absence of nutritional periodization in male professional soccer players. *International Journal of Sport Nutrition & Exercise Metabolism*, *33*(6), 349-359.

Chen, S., Scott, C., Pearce, J. V., Farrar, J. S., Evans, R. K., & Celi, F. S. (2020). An appraisal of whole-room indirect calorimeters and a metabolic cart for measuring resting and active metabolic rates. *Scientific Reports*, *10*(1), 1–8.

Christin-Maitre, S. (2013). History of oral contraceptive drugs and their use worldwide. *Best Practice and Research: Clinical Endocrinology and Metabolism*, *27*(1), 3–12.

Claessens-Van Ooijen, A. M. J., Westerterp, K. R., Wouters, L., Schoffelen, P. F. M., Van Steenhoven, A. A., & Van Marken Lichtenbelt, W. D. (2006). Heat production and body temperature during cooling and rewarming in overweight and lean men. *Obesity*, *14*(11), 1914–1920.

Coates, A. M., Incognito, A. V., Seed, J. D., Doherty, C. J., Millar, P. J., & Burr, J. F. (2018). Three weeks of overload training increases resting muscle sympathetic activity. *Medicine and Science in Sports and Exercise*, *50*(5), 928–937.

Collins, L. C., Cornelius, M. F., Vogel, R. L., Wallker, J. F., & Stamford, B. A. (1994). Effect of caffeine and/or cigarette smoking on resting energy expenditure. *International Journal of Obesity*, *18*(8), 551–556.

Collins, L. C., Walker, J., & Stamford, B. A. (1996). Smoking multiple high- versus low-nicotine cigarettes: Impact on resting energy expenditure. *Metabolism Clinical and Experimental*, *45*(8), 923–926.

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.

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.

Covassin, N., Singh, P., McCrady-Spitzer, S. K., St Louis, E. K., Calvin, A. D., Levine, J. A., & Somers, V. K. (2022). Effects of experimental sleep restriction on energy intake, energy expenditure, and visceral obesity. *Journal of the American College of Cardiology*, *79*(13), 1254–1265.

Cowley, E. S., Olenick, A. A., Mcnulty, K. L., & Ross, E. Z. (2021). "Invisible sportswomen": The sex data gap in sport and exercise science research. *Women in Sport & Physical Activity Journal*, *29*(2), 146–151.

Creswell, J. W., & Miller, D. L. (2000). Determining validity in qualitative inquiry. *Theory into Practice*, *39*(3), 124–130.

Cunha, F. A., Midgley, A. W., McNaughton, L. R., & Farinatti, P. T. V. (2016). Effect of continuous and intermittent bouts of isocaloric cycling and running exercise on excess

postexercise oxygen consumption. *Journal of Science and Medicine in Sport*, *19*(2), 187–192.

Cunningham, J. (1980). A reanalysis of the factors influencing basal metabolic rate in normal adults. *The American Journal of Clinical Nutrition*, *33*(11), 2372–2374.

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

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

Damask, M. C., Askanazi, J., Weissman, C., Elwyn, D. H., & Kinney, J. M. (1983). Artifacts in measurement of resting energy expenditure. *Critical Care Medicine*, *11*(9), 750–752.

Dauncey, M. J. (1980). Metabolic effects of altering the 24 h energy intake in man, using direct and indirect calorimetry. *British Journal of Nutrition*, *43*(2), 257–269.

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.

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.

De Souza, M. J., Hontscharuk, R., Olmsted, M., Kerr, G., & Williams, N. I. (2007). Drive for thinness score is a proxy indicator of energy deficiency in exercising women. *Appetite*, *48*(3), 359–367.

De Souza, M. J., Miller, B. E., Loucks, A. B., Luciano, A. A., Pescatello, L. S., Campbell, C. G., & Lasley, B. L. (1998). High frequency of luteal phase deficiency and anovulation in recreational women runners: Blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. *Journal of Clinical Endocrinology and Metabolism*, *83*(12), 4220–4232.

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.

Debevec, T. (2017). Hypoxia-related hormonal appetite modulation in humans during rest and exercise: Mini review. *Frontiers in Physiology*, *8*, 1–8.

Diaz, E. O., Prentice, A. M., Goldberg, G. R., Murgatroyd, P. R., & Coward, W. A. (1992). Metabolic response to experimental overfeeding in lean and overweight healthy volunteers. *American Journal of Clinical Nutrition*, *56*(4), 641–655.

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.

Dirlewanger, M., Di Vetta, V., Guenat, E., Battilana, P., Seematter, G., Schneiter, P., Jequier, E., & Tappy, L. (2000). Effects of short-term carbohydrate or fat overfeeding on energy expenditure and plasma leptin concentrations in healthy female subjects. *International Journal of Obesity*, *24*(11), 1413–1418.

Dolezal, B. A., Potteiger, J. A., Jacobsen, D. J., & Benedict, S. H. (2000). Muscle damage and resting metabolic rate after acute resistance exercise with an eccentric overload. *Medicine & Science in Sports & Exercise*, *32*(7), 1202–1207.

Doucet, E., St-Pierre, S., Alméras, N., Després, J.-P., Bouchard, C., & Tremblay, A. (2001). Evidence for the existence of adaptive thermogenesis during weight loss. *British Journal of Nutrition*, *85*(6), 715–723.

Doyle-Lucas, A. F., Akers, J. D., & Davy, B. M. (2010). Energetic efficiency, menstrual Irregularity, and bone mineral density in elite professional female ballet dancers. *Journal of Dance Medicine and Science*, *14*(4), 146–154.

Drenowatz, C., Eisenmann, J. C., Carlson, J. J., Pfeiffer, K. A., & Pivarnik, J. M. (2012). Energy expenditure and dietary intake during high-volume and low-volume training periods among male endurance athletes. *Applied Physiology, Nutrition and Metabolism*, *37*(2), 199–205.

Drenowatz, C., Eisenmann, J. C., Pivarnik, J. M., Pfeiffer, K. A., & Carlson, J. J. (2013). Differences in energy expenditure between high- and low-volume training. *European Journal of Sport Science*, *13*(4), 422–430.

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.

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.

Dulloo, A. G., Geissler, C. A., Horton, T., Collins, A., & Miller, D. S. (1989). Normal caffeine consumption: Influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. *American Journal of Clinical Nutrition*, *49*(1), 44–50.

Dupertuis, Y. M., Delsoglio, M., Hamilton-James, K., Berger, M. M., Pichard, C., Collet, T. H., & Genton, L. (2022). Clinical evaluation of the new indirect calorimeter in canopy and face mask mode for energy expenditure measurement in spontaneously breathing patients. *Clinical Nutrition*, *41*(7), 1591–1599.

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.

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.

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.

Elliott-Sale, K. J., Smith, S., Bacon, J., Clayton, D., McPhilimey, M., Goutianos, G., Hampson, J., & Sale, C. (2013). Examining the role of oral contraceptive users as an experimental and/or control group in athletic performance studies. *Contraception*, *88*(3), 408–412.

Færevik, H., Markussen, D., Øglænd, G. E., & Reinertsen, R. E. (2001). The thermoneutral zone when wearing aircrew protective clothing. *Journal of Thermal Biology*, *26*(4–5), 419–425.

Fahrenholtz, I. L., Sjödin, A., Benardot, D., Tornberg, B., Skouby, S., Faber, J., Sundgot-Borgen, J. K., & Melin, A. K. (2018). Within-day energy deficiency and reproductive function in female endurance athletes. *Scandinavian Journal of Medicine and Science in Sports*, *28*(3), 1139–1146.

FAO/WHO/UNU. (2001). *Human energy requirements: report of a joint FAO/WHO/UNU Expert Consultation.* https://www.fao.org/4/y5686e/y5686e00.htm

Ferrannini, E. (1988). The theoretical bases of indirect calorimetry: A review. *Metabolism*, *37*(3), 287–301.

Ferrara, P. J., Lang, M. J., Johnson, J. M., Watanabe, S., McLaughlin, K. L., Maschek, J. A., Verkerke, A. R. P., Riripoksup, P., Chaix, A., Fisher-Wellman, K. H., & Funai, K. (2023). Weight loss increases skeletal muscle mitochondrial energy efficiency in obese mice. *Life Metabolism*, *2*(2), load014.

Ferraro, R., Lillioja, S., Fontvieille, A. M., Rising, R., Bogardus, C., & Ravussin, E. (1992). Lower sedentary metabolic rate in women compared with men. *Journal of Clinical Investigation*, *90*(3), 780–784.

Fischer, M. A. (2008). Implanon: A new contraceptive implant. *JOGNN - Journal of Obstetric, Gynecologic, and Neonatal Nursing*, *37*(3), 361–368.

Flaherty, G., O'Connor, R., & Johnston, N. (2016). Altitude training for elite endurance athletes: A review for the travel medicine practitioner. *Travel Medicine and Infectious Disease*, *14*(3), 200–211.

Flatt, A. A., Hornikel, B., & Esco, M. R. (2017). Heart rate variability and psychometric responses to overload and tapering in collegiate sprint-swimmers. *Journal of Science and Medicine in Sport*, *20*(6), 606–610.

Flueck, J. L., & Koehler, K. (2022). Case Study: Improving energy status in a wheelchair athlete with suppressed resting energy expenditure. *International Journal of Sports Physiology and Performance*, *17*(7), 1151–1154.

Fogt, D. L., Levi, M. A., Rickards, C. A., Stelly, S. P., & Cooke, W. H. (2016). Effects of acute vaporized nicotine in non-tobacco users at rest and during exercise. *International Journal of Exercise Science*, *9*(5), 607–615.

Forse, R. A. (1993). Comparison of gas exchange measurements with a mouthpiece, face mask, and ventilated canopy. *Journal of Parenteral and Enteral Nutrition*, *17*(4), 388–391.

Fothergill, E., Guo, J., Howard, L., Kerns, J. C., Knuth, N. D., Brychta, R., Chen, K. Y., Skarulis, M. C., Walter, M., Walter, P. J., & Hall, K. D. (2016). Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. *Obesity*, *24*(8), 1612–1619.

Francois, M. E., Graham, M. J., Parr, E. B., Rehrer, N. J., Lucas, S. J. E., Stavrianeas, S., & Cotter, J. D. (2017). Similar metabolic response to lower- versus upper-body interval exercise or endurance exercise. *Metabolism*, *68*, 1–10.

Frankenfield, D. C., & Coleman, A. (2009). Recovery to resting metabolic state after walking. *Journal of the American Dietetic Association*, *109*(11), 1914–1916.

Frankenfield, D. C., & Coleman, A. (2013). An evaluation of a handheld indirect calorimeter against a standard calorimeter in obese and nonobese adults. *Journal of Parenteral and Enteral Nutrition*, *37*(5), 652–658.

Freire, R., Alcantara, J. M. A., Hausen, M., & Itaborahy, A. (2021). The estimation of the resting metabolic rate is affected by the method of gas exchange data selection in high-level athletes. *Clinical Nutrition ESPEN*, *41*, 234–241.

Frisard, M. I., Broussard, A., Davies, S. S., Roberts, L. J., Rood, J., De Jonge, L., Fang, X., Jazwinski, S. M., Deutsch, W. A., & Ravussin, E. (2007). Aging, resting metabolic rate, and oxidative damage: Results from the Louisiana healthy aging study. *The Journals of Gerontology- Series A Biological Sciences and Medical Sciences*, *62*(7), 752–759.

Fukagawa, N. K., Bandini, L. G., & Young, J. B. (1990). Effect of age on body composition and resting metabolic rate. *American Journal of Physiology*, *259*(2 Pt 1), E233-8.

Fullmer, S., Benson-Davies, S., Earthman, C. P., Frankenfield, D. C., Gradwell, E., Lee, P. S. P., Piemonte, T., & Trabulsi, J. (2015). Evidence analysis library review of best practices for performing indirect calorimetry in healthy and non-critically ill individuals. *Journal of the Academy of Nutrition and Dietetics*, *115*(9), 1417-1446.e2.

Gallagher, D., Albu, J., He, Q., Heshka, S., Boxt, L., Krasnow, N., & Elia, M. (2006). Small organs with a high metabolic rate explain lower resting energy expenditure in African American than in white adults. *American Journal of Clinical Nutrition*, *83*(5), 1062–1067.

Gallagher, D., Allen, A., Wang, Z., Heymsfield, S. B., & Krasnow, N. (2000). Smaller organ tissue mass in the elderly fails to explain lower resting metabolic rate. *Annals of the New York Academy of Sciences*, *904*, 449–455.

Gannon, B., Dipietro, L., & Poehlman, E. T. (2000). Do African Americans have lower energy expenditure than Caucasians? *International Journal of Obesity*, *24*, 4–13.

Gardiner, C., Weakley, J., Burke, L. M., Roach, G. D., Sargent, C., Maniar, N., Townshend, A., & Halson, S. L. (2023). The effect of caffeine on subsequent sleep: A systematic review and meta-analysis. *Sleep Medicine Reviews*, *69*, 101764.

Garn, S. M., & Clark, L. C. (1953). The sex difference in the basal metabolic rate. *Child Development*, *24*(4), 215–224.

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.

Geer, E. B., & Shen, W. (2009). Gender differences in insulin resistance, body composition, and energy balance. *Gender Medicine*, *6*(1), 60–75.

Geisler, C., Braun, W., Pourhassan, M., Schweitzer, L., Glüer, C. C., Bosy-Westphal, A., & Müller, M. J. (2016a). Age-dependent changes in resting energy expenditure (REE): Insights from detailed body composition analysis in normal and overweight healthy caucasians. *Nutrients*, *8*(6), 322.

Geisler, C., Braun, W., Pourhassan, M., Schweitzer, L., Glüer, C. C., Bosy-Westphal, A., & Müller, M. J. (2016b). Gender-specific associations in age-related changes in resting energy expenditure (REE) and MRI measured body composition in healthy caucasians. *The Journals of Gerontology: Medical Sciences*, *71*(7), 941–946.

Gillette, C. A., Bullough, R. C., & Melby, C. L. (1994). Postexercise energy expenditure in response to acute aerobic or resistive exercise. *International Journal of Sport Nutrition*, *4*(4), 347–360.

Gilliat-Wimberly, M., Manore, M.M., Woolf, K., Swan, P.D., Carroll, S. S. (2001). Effects of habitual physical activity on the resting metabolic rates and body compositions of women aged 35 to 50 years. *Journal of the American Dietetic Association*, *101*(10), 1181–1188.

Going, S. B., Massett, M. P., Hall, M. C., Bare, L. A., Root, P. A., Williams, D. P., & Lohman, T. G. (1993). Detection of small changes in body composition by dual-energy x-ray absorptiometry. *American Journal of Clinical Nutrition*, *57*(6), 845–850.

Gonnissen, H. K. J., Rutters, F., Mazuy, C., Martens, E. A. P., Adam, T. C., & Westerterp-Plantenga, M. S. (2012). Effect of a phase advance and phase delay of the 24-h cycle on energy metabolism, appetite, and related hormones. *American Journal of Clinical Nutrition*, *96*(4), 689–697.

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.

Gore, C. J., Clark, S. A., & Saunders, P. U. (2007). Nonhematological mechanisms of improved sea-level performance after hypoxic exposure. *Medicine and Science in Sports and Exercise*, *39*(9), 1600–1609.

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 Sport and Exercise*, *53*(11), 2396–2404.

Grant, L. K., Coborn, J. E., Cohn, A., Nathan, M. D., Scheer, F. A. J. L., Klerman, E. B., Kaiser, U. B., Harder, J., Abramson, M., Elguenaoui, E., Russell, J. A., Wiley, A., Rahman, S. A., & Joffe, H. (2022). Sleep fragmentation and estradiol suppression decrease fat oxidation in premenopausal women. *Journal of Clinical Endocrinology and Metabolism*, *107*(8), E3167–E3176.

Grant, S. S., Magruder, K. P., & Friedman, B. H. (2018). Controlling for caffeine in cardiovascular research: A critical review. *International Journal of Psychophysiology*, *133*, 193–201.

Greer, B. K., O'brien, J., Hornbuckle, L. M., & Panton, L. B. (2021). EPOC comparison between resistance training and high-intensity interval training in aerobically fit women. *International Journal of Exercise Science*, *14*(2), 1027–1035.

Greer, B. K., Sirithienthad, P., Moffatt, R. J., Marcello, R. T., & Panton, L. B. (2015). EPOC comparison between isocaloric bouts of steady-state aerobic, intermittent aerobic, and resistance training. *Research Quarterly for Exercise and Sport*, *86*(2), 190–195.

Grover, R. F. (1963). Basal oxygen uptake of man at high altitude. *Journal of Applied Physiology*, *18*(5), 909–912.

Guebels, C. P., Kam, L. C., Maddalozzo, G. F., & Manore, M. M. (2014). Active women before/after an intervention designed to restore menstrual function: Resting metabolic rate and comparison of four methods to quantify energy expenditure and energy availability. *International Journal of Sport Nutrition and Exercise Metabolism*, *24*(1), 37–46.

Hackney, K. J., Engels, H. J., & Gretebeck, R. J. (2008). Resting energy expenditure and delayed-onset muscle soreness after full-body resistance training with an eccentric concentration. *Journal of Strength and Conditioning Research*, *22*(5), 1602–1609.

Haggarty, P., McNeill, G., Manneh, M. K. A., & Davidson, L. (1994). The influence of exercise on the energy requirements of adult males in the UK. *British Journal of Nutrition*, *72*(6), 799–813.

Halson, S. L., & Jeukendrup, A. E. (2004). Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Medicine*, *34*(14), 967–981.

Hamada, T., Kotani, K., Higashi, A., Ikeda, J., Tagaki, E., Igarashi, A., Matsubara, C., Yoshida, T., & Sakane, N. (2008). Lack of association of the Trp64Arg polymorphism of β-3-adrenergic receptor gene with energy expenditure in response to caffeine among young healthy women. *Tohoku Journal of Experimental Medicine*, *214*(4), 365–370.

Hannon, J. P., & Sudman, D. M. (1973). Basal metabolic and cardiovascular function of women during altitude acclimatization. *Journal of Applied Physiology*, *34*(4), 471–477.

Harris, A. M., Jensen, M. D., & Levine, J. A. (2006). Weekly changes in basal metabolic rate with eight weeks of overfeeding. *Obesity*, *14*(4), 690–695.

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.

Haugen, A. H., Chan, L. N., & Li, F. (2007). Indirect calorimetry: A practical guide for clinicians. *Nutrition in Clinical Practice*, *22*(4), 377–388.

Haugen, H. A., Melanson, E. L., Tran, Z. V., Kearney, J. T., & Hill, J. O. (2003). Variability of measured resting metabolic rate. *American Journal of Clinical Nutrition*, *78*, 1141–1145.

Hayes, M., Chustek, M., Wang, Z. M., Gallagher, D., Heshka, S., Spungen, A., Bauman, W., & Heymsfield, S. B. (2002). DXA: Potential for creating a metabolic map of organ-tissue resting energy expenditure components. *Obesity Research*, *10*(10), 969–977.

Hellerstein, M. K., Benowitz, N. L., Neese, R. A., Schwartz, J. M., Hoh, R., Jacob, P., Hsieh, J., & Faix, D. (1994). Effects of cigarette smoking and its cessation on lipid metabolism and energy expenditure in heavy smokers. *Journal of Clinical Investigation*, *93*(1), 265–272.

Henderson, M. E. T., Brayson, D., & Halsey, L. G. (2021). The cardio-respiratory effects of passive heating and the human thermoneutral zone. *Physiological Reports*, *9*(16), 1–12.

Henriksen, H. B., Henriksen, C., de Sousa, A. R. S., Alavi, D. T., Augestad, E. M. S., Rising, R., Dörner, R., Hägele, F. A., Bosy-Westphal, A., Blomhoff, R., Ulven, S. M., & Olsen, T. (2023). Validity and reproducibility of a whole-room indirect calorimeter for the estimation of VO2, VCO2, and resting metabolic rate. *Physiological Reports*, *11*(7), 1–9.

Herring, J. L., Mole, P. A., Meredith, C. N., & Stern, J. S. (1991). Effect of suspending exercise training on resting metabolic rate in women. *Medicine and Science in Sports & Exercise*, *42*(1), 59–65.

Heydenreich, J., Kayser, B., Schutz, Y., & Melzer, K. (2017). Total energy expenditure, energy intake, and body composition in endurance athletes across the training season: A systematic review. *Sports Medicine - Open*, *3*(1), 1–24.

Heymsfield, S. B., Gallagher, D., Kotler, D. P., Wang, Z., Allison, D. B., & Heshka, S. (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–E138

Heymsfield, S. B., Smith, B., Dahle, J., Kennedy, S., Fearnbach, N., Thomas, D. M., Bosy-Westphal, A., & Müller, M. J. (2021). Resting energy expenditure: From cellular to whole-body level, a mechanistic historical perspective. *Obesity*, *29*(3), 500–511.

Heymsfield, S. B., Thomas, D. M., Bosy-Westphal, A., & Muller, M. J. (2019). The anatomy of resting energy expenditure: Body composition mechanisms. *European Journal of Clinical Nutrition*, *73*(2), 166–171.

Hill, J. O., Heymsfield, S. B., McMannus, C., & DiGirolamo, M. (1984). Meal size and thermic response to food in male subjects as a function of maximum aerobic capacity. *Metabolism*, *33*(8), 743–749.

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.

Hofstetter, A., Schutz, Y., Jéquier, E., & Wahren, J. (1986). Increased 24-hour energy expenditure in cigarette smokers. *The New England Journal of Medicine*, *314*(2), 79–82.

Hollands, M. A., Arch, J. R. S., & Cawthorne, M. A. (1981). A simple apparatus for comparative measurements of energy expenditure in human subjects: The thermic effect of caffeine. *American Journal of Clinical Nutrition*, *34*(10), 2291–2294.

Holliday, M. A. (1971). Metabolic rate and organ size during growth from infancy to maturity and during late gestation and early infancy. *Pediatrics*, *47*(1), 169–179.

Howe, J. C., Rumpler, W. V., & Seale, J. L. (1993). Energy expenditure by indirect calorimetry in premenopausal women: variation within one menstrual cycle. *The Journal of Nutritional Biochemistry*, *4*(5), 268–273.

Hsu, A., Heshka, S., Janumala, I., Song, M. Y., Horlick, M., Krasnow, N., & Gallagher, D. (2003). Larger mass of high-metabolic-rate organs does not explain higher resting energy expenditure in children. *The American Journal of Clinical Nutrition*, *77*(6), 1506–1511.

Huang, S. Y., Alexander, J. K., Grover, R. F., Maher, J. T., McCullough, R. E., McCullough, R. G., Moore, L. G., Weil, J. V., Sampson, J. B., & Reeves, J. T. (1984). Increased metabolism contributes to increased resting ventilation at high altitude. *Respiration Physiology*, *57*(3), 377–385.

Hudson, J. F., Cole, M., Morton, J. P., Stewart, C. E., & Close, G. L. (2020). Daily changes of resting metabolic rate in elite rugby union players. *Medicine and Science in Sports and Exercise*, *52*(3), 637–644.

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

Hunter, G. R., Moellering, D. R., Carter, S. J., Gower, B. A., Bamman, M. M., Hornbuckle, L. M., Plaisance, E. P., & Fisher, G. (2017). Potential causes of elevated REE following high-intensity exercise. *Medicine and Science in Sports and Exercise*, *49*(12), 2414–2421.

Hunter, G. R., Weinsier, R. L., Gower, B. A., & Wetzstein, C. (2001). Age-related decrease in resting energy expenditure in sedentary white women: Effects of regional differences in lean and fat mass. *American Journal of Clinical Nutrition*, *73*(2), 333–337.

Hunter, G. R., Weinsier, R. L., Darnell, B. E., Zuckerman, P. A., & Goran, M. I. (2000). Racial differences in energy expenditure and aerobic fitness in premenopausal women. *American Journal of Clinical Nutrition*, *71*(2), 500–506.

Hutson, M. J., O'Donnell, E., McConnell, K., Chauntry, A. J., & Blagrove, R. C. (2024). Energy availability modulates regional blood flow via estrogen-independent pathways in regularly menstruating young women. *European Journal of Applied Physiology, 124*(10), 3045-3055.

Iraki, J., Paulsen, G., Garthe, I., Slater, G., & Areta, J. L. (2023). Reliability of resting metabolic rate between and within day measurements using the Vyntus CPX system and comparison against predictive formulas. *Nutrition and Health*, *29*(1), 107–114.

Irving, C. J., Eggett, D. L., & Fullmer, S. (2017). Comparing steady state to time interval and non-steady state measurements of resting metabolic rate. *Nutrition in Clinical Practice*, *32*(1), 77–83.

Isbell, T. R., Klesges, R. C., Meyers, A. W., & Klesges, L. M. (1991). Measurement reliability and reactivity using repeated measurements of resting energy expenditure with a face mask, mouthpiece, and ventilated canopy. *Journal of Parenteral and Enteral Nutrition*, *15*(2), 165–168.

Jagim, A. R., Camic, C. L., Askow, A., Lueke, J., Erickson, J., Kerksick, C. M., Jones, M. T., & Oliver, J. M. (2019). Sex differences in resting metabolic rate among athletes. *Journal of Strength & Conditioning Research*, *33*(11), 3008–3014.

Jagim, A. R., Camic, C. L., Kisiolek, J., Luedke, J., Erickson, J., Jones, M. T., & Oliver, J. M. (2018). Accuracy of resting metabolic rate prediction equations in athletes. *Journal of Strength & Conditioning Research*, *32*(7), 1875–1881.

Jagim, A. R., Jones, M. T., Askow, A. T., Luedke, J., Erickson, J. L., Fields, J. B., & Kerksick, C. M. (2023). Sex differences in resting metabolic rate among athletes and association with body composition parameters: A follow-up investigation. *Journal of Functional Morphology and Kinesiology*, *8*(3), 109.

Jamurtas, A. Z., Koutedakis, Y., Paschalis, V., Tofas, T., Yfanti, C., Tsiokanos, A., Koukoulis, G., Kouretas, D., & Loupos, D. (2004). The effects of a single bout of exercise on resting energy expenditure and respiratory exchange ratio. *European Journal of Applied Physiology*, *92*(4–5), 393–398.

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.

Jéquier, E. (2002). Pathways to obesity. *International Journal of Obesity*, *26*(2), S12–S17.

Jetté, M., Sidney, K., & Blümchen, G. (1990). Metabolic equivalents (METS) in exercise testing, exercise prescription, and evaluation of functional capacity. *Clinical Cardiology*, *13*(8), 555–565.

Johnstone, A. M., Murison, S. D., Duncan, J. S., Rance, K. A., & Speakman, J. R. (2005). Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *American Journal of Clinical Nutrition*, *82*(5), 941–948.

Jones, A. J., Shen, W., Gallagher, D., Heshka, S., Wang, Z., & Heymsfield, S. B. (2004). Body-composition differences between African American and white women: relation to resting energy requirements. *The American Journal of Clinical Nutrition*, *79*(5), 780–786.

Joosen, A. M. C. P., Bakker, A. H. F., & Westerterp, K. R. (2005). Metabolic efficiency and energy expenditure during short-term overfeeding. *Physiology & Behavior*, *85*(5), 593–597.

Júdice, P. B., Magalhães, J. P., Santos, D. A., Matias, C. N., Carita, A. I., Armada-Da-Silva, P. A. S., Sardinha, L. B., & Silva, A. M. (2013). A moderate dose of caffeine ingestion does not change energy expenditure but decreases sleep time in physically active males: A double-blind randomized controlled trial. *Applied Physiology, Nutrition and Metabolism*, *38*(1), 49–56.

Jung, R. T., Shetty, P. S., James, W. P. T., Barrand, M. A., & Callingham, B. A. (1981). Caffeine: its effect on catecholamines and metabolism in lean and obese humans. *Clinical Science*, *60*(5), 527–535.

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.

Jurov, I., Hadžić, V., & Rauter, S. (2020). Markers of energy metabolism affect lactate metabolism and aerobic performance in competitive female cyclists. *Applied Sciences (Switzerland)*, *10*(21), 1–11.

Jurov, I., Keay, N., Hadžić, V., Spudić, D., & Rauter, S. (2021). Relationship between energy availability, energy conservation and cognitive restraint with performance measures in male endurance athletes. *Journal of the International Society of Sports Nutrition*, *18*(1), 1–11.

Jurov, I., Keay, N., & Rauter, S. (2022). Reducing energy availability in male endurance athletes: a randomized trial with a three-step energy reduction. *Journal of the International Society of Sports Nutrition*, *19*(1), 179–195.

Jurov, I., Keay, N., Spudić, D., & Rauter, S. (2022). Inducing low energy availability in trained endurance male athletes results in poorer explosive power. *European Journal of Applied Physiology*, *122*(2), 503–513.

Kaneko, K., Ito, C., Koizumi, K., Watanabe, S., Umeda, Y., & Ishikawa-Takata, K. (2013). Resting energy expenditure (REE) in six- to seventeen-year-old Japanese children and adolescents. *Journal of Nutritional Science and Vitaminology*, *59*(4), 299–309.

Kang, B. S., Song, S. H., Suh, C. S., Hong, S. K., Soon, B., Hoon Song, S., Sam Suh, C., & Hong, S. K. (1963). Changes in body temperature and basal metabolic rate of the amal. *Journal of Applied Physiology*, *18*(3), 483–488.

Kang, J., Hasan, S. B., Ellis, N. A., Vought, I. T., Ratamess, N. A., Bush, J. A., & Faigenbaum, A. D. (2020). Effects of exercise with and without energy replacement on substrate utilization in the fasting state. *Journal of the American College of Nutrition*, *39*(1), 39–46.

Kashiwazaki, H., Dejima, Y., & Suzuki, T. (1990). Influence of upper and lower thermoneutral room temperature (20 degrees C and 25 degrees C) on fasting and post-prandial resting metabolism under different outdoor temperatures. *European Journal of Clinical Nutrition*, *44*(5), 405–413.

Kaufman, B. A., Warren, M. P., Dominguez, J. E., Wang, J., Heymsfield, S. B., & Pierson, R. N. (2002). Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels. *Journal of Clinical Endocrinology and Metabolism*, *87*(6), 2777–2783.

Kennedy, S., Ryan, L., Fraser, A., & Clegg, M. E. (2014). Comparison of the GEM and the ECAL indirect calorimeters against the Deltatrac for measures of RMR and diet-induced thermogenesis. *Journal of Nutritional Science, 7*(3), e52.

Kenny, G. P., Notley, S. R., & Gagnon, D. (2017). Direct calorimetry: a brief historical review of its use in the study of human metabolism and thermoregulation. *European Journal of Applied Physiology*, *117*(9), 1765–1785.

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.

Kinoshita, N., Uchiyama, E., Ishikawa-Takata, K., Yamada, Y., & Okuyama, K. (2021). Association of energy availability with resting metabolic rates in competitive female teenage runners: a cross-sectional study. *Journal of the International Society of Sports Nutrition*, *18*(1), 70.

Kirkby, J., Metcalf, B. S., Jeffery, A. N., O'riordan, C. F., Perkins, J., Voss, L. D., & Wilkin, T. J. (2004). Sex differences in resting energy expenditure and their relation to insulin resistance in children (EarlyBird 13). *American Journal of Clinical Nutrition, 80(*2), 430-5.

Klausen, B., Toubro, S., & Astrup, A. (1997). Age and sex effects on energy expenditure. *American Journal of Clinical Nutrition*, *65*(4), 895–907.

Kleiber, M. (1947). Body size and metabolic rate. *Physiological Reviews*, *27*(4), 511–541.

Klesges, R. C., DePue, K., Audrain, J., Klesges, L. M., & Meyers, A. W. (1991). Metabolic effects of nicotine gum and cigarette smoking: Potential implications for postcessation weight gain? *Journal of Consulting and Clinical Psychology*, *59*(5), 749–752.

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.

Kojima, C., Ishibashi, A., Tanabe, Y., Iwayama, K., Kamei, A., Takahashi, H., & Goto, K. (2020). Muscle glycogen content during endurance training under low energy availability. *Medicine and Science in Sports and Exercise*, *52*(1), 187–195.

Kolkhorst, F. W., Londeree, B. R., & Thomas, T. R. (1994). Effects of consecutive exercise days of jogging or cycling on the resting metabolic rate and nitrogen balance. *Journal of Sports Medicine and Physical Fitness*, *34*(4), 343–350.

Koot, P., & Deurenberg, P. (1995). Comparison of changes in energy expenditure and body temperatures after caffeine consumption. *Annals of Nutrition and Metabolism*, *39*(3), 135–142.

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.

Krems, C., Lührmann, P. M., Strassburg, A., Hartmann, B., & Neuhäuser-Berthold, M. (2005). Lower resting metabolic rate in the elderly may not be entirely due to changes in body composition. *European Journal of Clinical Nutrition*, *59*(2), 255–262.

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.

Kuikman, M. A., Coates, A. M., & Burr, J. F. (2022). Markers of low energy availability in overreached athletes: A systematic review and meta-analysis. *Sports Medicine*, *52*(12), 2925–2941.

Kuikman, M. A., McKay, A. K. A., Minahan, C., Harris, R., Elliott-Sale, K. J., Stellingwerff, T., Smith, E. S., McCormick, R., Tee, N., Skinner, J., Ackerman, K. E., & Burke, L. M. (2024). Effect of menstrual cycle phase and hormonal contraceptives on resting metabolic rate and body composition. *International Journal of Sport Nutrition and Exercise Metabolism*, *34*(4), 207-217.

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.

Kuikman, M. A., Smith, E., McKay, A. K. A., McCormick, R., Ackerman, K. E., Harris, R., Elliott-Sale, K. J., Stellingwerff, T., & Burke, L. M. (2024). Impact of acute dietary and exercise manipulation on next day RMR measurements and DXA body composition estimates. *Medicine & Science in Sports & Exercise*. Online ahead of print.

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.

Kumahara, H., Yoshioka, M., Yoshitake, Y., Shindo, M., Schutz, Y., & Tanaka, H. (2004). The difference between the basal metabolic rate and the sleeping metabolic rate in Japanese. *Journal of Nutritional Science and Vitaminology*, *50*(6), 441–445.

Kush, R. D., Young, J. B., Katzeff, H. L., Danforth, E., Garrow, J. S., Scheidegger, K., Ravussin, E., Howard, B. V, Sims, E. A. H., Horton, E. S., & Landsberg, L. (1986). Effect of diet on energy expenditure and plasma norepinephrine in lean and obese Pima Indians. *Metabolism*, *35*(12), 1110–1120.

Kushner, R. F., Racette, S. B., Neil, K., & Schoeller, D. A. (1995). Measurement of physical activity among black and white obese women. *Obesity Research*, *3*(2), 261s–265s.

Laforgia, J., Withers, R. T., & Gore, C. J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *Journal of Sports Sciences*, *24*(12), 1247–1264.

LaForgia, J., Withers, R. T., Williams, A. D., Murch, B. J., Chatterton, B. E., Schultz, C. G., & Leaney, F. (1999). Effect of 3 weeks of detraining on the resting metabolic rate and body composition of trained males. *European Journal of Clinical Nutrition*, *53*(2), 126–133.

Lane, J. D., Steege, J. F., Rupp, S. L., & Kuhn, C. M. (1992). Menstrual cycle effects on caffeine elimination in the human female. *European Journal of Clinical Pharmacology, 43*(5), 543–546.

Langan-Evans, C., Germaine, M., Artukovic, M., Oxborough, D. L., Areta, J. L., Close, G. L., & Morton, J. P. (2021). The psychological and physiological consequences of low energy availability in a male combat sport athlete. *Medicine & Science in Sports & Exercise*, *53*(4), 673–683.

Larsen, F. J., Schiffer, T. A., Sahlin, K., Ekblom, B., Weitzberg, E., & Lundberg, J. O. (2011). Mitochondrial oxygen affinity predicts basal metabolic rate in humans. *The FASEB Journal*, *25*(8), 2843–2852.

Le Meur, Y., Pichon, A., Schaal, K., Schmitt, L., Louis, J., Gueneron, J., Vidal, P. P., & Hausswirth, C. (2013). Evidence of parasympathetic hyperactivity in functionally overreached athletes. *Medicine and Science in Sports and Exercise*, *45*(11), 2061–2071.

Leal-Martín, J., Mañas, A., Alfaro-Acha, A., García-García, F. J., & Ara, I. (2023). Optimization of VO2 and VCO2 outputs for the calculation of resting metabolic rate using a portable indirect calorimeter. *Scandinavian Journal of Medicine and Science in Sports*, *33*(9), 1648–1660.

Lebenstedt, M., Platte, P., & Pirke, K. M. (1999). Reduced resting metabolic rate in athletes with menstrual disorders. *Medicine & Science in Sports & Exercise*, *31*(9), 1250–1256.

LeBlanc, J., Jobin, M., Cote, J., Samson, P., & Labrie, A. (1985). Enhanced metabolic response to caffeine in exercise-trained human subjects. *Journal of Applied Physiology*, *59*(3), 832–837.

Lee, S., Kuniko, M., Han, S., Oh, T., & Taguchi, M. (2020). Association of low energy availability and suppressed metabolic status in Korean male collegiate soccer players: A pilot study. *American Journal of Men's Health*, *14*(6), 1–9.

Leff, M. L., Hill, J. O., Yates, A. A., Cotsonis, G. A., & Heymsfield, S. B. (1987). Resting metabolic rate: Measurement reliability. *Journal of Parenteral and Enteral Nutrition*, *11*(4), 354–359.

Leibel, R. L., Rosenbaum, M., & Hirsch, J. (1995). Changes in energy expenditure resulting from altered body weight. *The New England Journal of Medicine*, *332*(10), 621–628.

Leonard, W. R., Levy, S. B., Tarskaia, L. A., Klimova, T. M., Fedorova, V. I., Baltakhinova, M. E., Krivoshapkin, V. G., & Snodgrass, J. J. (2014). Seasonal variation in basal metabolic rates among the yakut (Sakha) of Northeastern Siberia. *American Journal of Human Biology*, *26*(4), 437–445.

Levine, J. A. (2005). Measurement of energy expenditure. *Public Health Nutrition*, *8*(7a), 1123–1132.

Littlewood, R. A., White, M. S., Bell, K. L., Davies, P. S. W., Cleghorn, G. J., & Grote, R. (2002). Comparison of the Cosmed K4 b2 and the Deltatrac II^{TM} metabolic cart in measuring resting energy expenditure in adults. *Clinical Nutrition*, *21*(6), 491–497.

Liu, A. G., Arceneaux, K. P., Chu, J. T., Jacob, G., Schreilber, A. L., Tipton, R. C., Yu, Y., Johnson, W. D., Greenway, F. L., & Primeaux, S. D. (2015). The effect of caffeine and albuterol on body composition and metabolic rate. *Obesity*, *23*(9), 1830–1835.

Löfberg, I. E., Karppinen, J. E., Laatikainen-Raussi, V., Lehti, M., Hackney, A. C., Ihalainen, J. K., & Mikkonen, R. S. (2024). Resting energy expenditure, metabolic and sex hormones in two phases of the menstrual and hormonal contraceptive cycles. *Medicine and Science in Sports and Exercise*. Online ahead of print.

Loucks, A. B., Kiens, B., & Wright, H. H. (2011). Energy availability in athletes. *Journal of Sports Sciences*, *29*(SUPPL. 1), S7-15.

Lührmann, P. M., Bender, R., Edelmann-Schäfer, B., & Neuhäuser-Berthold, M. (2009). Longitudinal changes in energy expenditure in an elderly German population: A 12-year follow-up. *European Journal of Clinical Nutrition*, *63*(8), 986–992.

Mackay, K. J., Schofield, K. L., Sims, S. T., Mcquillan, J. A., & Driller, M. W. (2019). The validity of resting metabolic rate-prediction equations and reliability of measured RMR in female athletes. *International Journal of Exercise Science*, *12*(2), 886–897.

MacKenzie-Shalders, K. L., Byrne, N. M., King, N. A., & Slater, G. J. (2019). Are increases in skeletal muscle mass accompanied by changes to resting metabolic rate in rugby athletes over a pre-season training period? *European Journal of Sport Science*, *19*(7), 885–892.

Maehlum, S., Grandmontagne, M., Newsholme, E. A., & Sejersted, O. M. (1986). Magnitude and duration of excess postexercise oxygen consumption in healthy young subjects. *Metabolism*, *35*(5), 425–429.

Mallinson, R. J., Williams, N. I., Olmsted, M. P., Scheid, J. L., Riddle, E. S., & De Souza, M. J. (2013). A case report of recovery of menstrual function following a nutritional intervention in two exercising women with amenorrhea of varying duration. *Journal of the International Society of Sports Nutrition*, *10*, 34.

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*, *131*(3), 384-390.

Manore, M. M. (2021). Energy requirements and measurement of energy expenditure. In L. M. Burke, V. Beakin, & M. Minehan (Eds.), *Clinical Sports Nutrition* (6th ed., pp. 90–99). McGraw Hill Education.

Marclay, F., Grata, E., Perrenoud, L., & Saugy, M. (2011). A one-year monitoring of nicotine use in sport: Frontier between potential performance enhancement and addiction issues. *Forensic Science International*, *213*(1–3), 73–84.

Margaria, R., Cerretelli, P., Aghemo, P., & Sassi, G. (1963). Energy cost of running. *Journal of Applied Physiology*, *18*(2), 367–370.

Martin, A., Fox, D., Murphy, C. A., Hofmann, H., & Koehler, K. (2022). Tissue losses and metabolic adaptations both contribute to the reduction in resting metabolic rate following weight loss. *International Journal of Obesity*, *46*, 1168–1175.

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.

Martin, K., Wallace, P., Rust, P. F., & Garvey, W. T. (2004). Estimation of Resting Energy Expenditure Considering Effects of Race and Diabetes Status. *Diabetes Care*, *27*(6), 1405–1411.

Mason, E. D., Jacob, M., & Balakrishnan, V. (1964). Racial group differences in the basal metabolism and body composition of Indian and European Women in Bombay. *Human Biology*, *36*(4), 374–396.

Maury-Sintjago, E., Rodríguez-Fernández, A., Parra-Flores, J., & Ruíz-De la Fuente, M. (2022). Obese women have a high carbohydrate intake without changes in the resting metabolic rate in the luteal phase. *Nutrients*, *14*(10), 1–8.

Mawson, J. T., Braun, B., Rock, P. B., Moore, L. G., Mazzeo, R., & Butterfield, G. E. (2000). Women at altitude: energy requirement at 4,300 m. *Journal of Applied Physiology*, *88*(1), 272–281.

McAnena, O. J., Harvey, L. P., Katzeff, H. L., & Daly, J. M. (1986). Indirect calorimetry: comparison of hood and mask systems for measuring resting energy expenditure in healthy volunteers. *Journal of Parenteral and Enteral Nutrition*, *10*(6), 555–557.

McHill, A. W., Melanson, E. L., Higgins, J., Connick, E., Moehlman, T. M., Stothard, E. R., & Wright, K. P. (2014). Impact of circadian misalignment on energy metabolism during simulated nightshift work. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(48), 17302–17307.

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*, *56*(4), 706-716.

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.

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.

McNeil, G., Bruce, A. C., Ross, E., & James, W. P. T. (1988). Energy balance in women using oral contraceptives. *Proceedings of the Nutrition Society*, *47*, 58A.

Melanson, E. L., Coelho, L. B., Tran, Z. V., Haugen, H. A., Kearney, J. T., & Hill, J. O. (2004). Validation of the BodyGem™ hand-held calorimeter. *International Journal of Obesity*, *28*(11), 1479–1484.

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.

Melby, C. L., Paris, H. L., Drew Sayer, R., Bell, C., & Hill, J. O. (2019). Increasing energy flux to maintain diet-induced weight loss. *Nutrients*, *11*(10), 2533.

Melby, C., Scholl, C., Edwards, G., & Bullough, R. (1993). Effect of acute resistance exercise on postexercise energy expenditure and resting metabolic rate. *Journal of Applied Physiology*, *75*(4), 1847–1853.

Melin, A. K., Heikura, I. A., Tenforde, A., & Mountjoy, M. (2019). Energy availability in athletics: Health, performance, and physique. *International Journal of Sport Nutrition and Exercise Metabolism*, *29*(2), 152–164.

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.

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.

Metsios, G. S., Flouris, A. D., Jamurtas, A. Z., Carrillo, A. E., Kouretas, D., Germenis, A. E., Gourgoulianis, K., Kiropoulos, T., Tzatzarakis, M. N., Tsatsakis, A. M., & Koutedakis, Y. (2007). Brief report: A brief exposure to moderate passive smoke increases metabolism and thyroid hormone secretion. *Journal of Clinical Endocrinology and Metabolism*, *92*(1), 208–211.

Metz, L., Isacco, L., & Redman, L. M. (2022). Effect of oral contraceptives on energy balance in women: A review of current knowledge and potential cellular mechanisms. *Metabolism*, *126*, 154919.

Michie, S., van Stralen, M. M., & West, R. (2011). The behaviour change wheel: A new method for characterising and designing behaviour change interventions. *Implementation Science*, *6*, 42.

Mihm, M., Gangooly, S., & Muttukrishna, S. (2011). The normal menstrual cycle in women. *Animal Reproduction Science*, *124*(3–4), 229–236.

Miles, C. W., Wong, N. P., Rumpler, W. V., & Conway, J. (1993). Effect of circadian variation in energy expenditure, within-subject variation and weight reduction on thermic effect of food. *European Journal of Clinical Nutrition*, *47*(4), 274–284.

Moffatt, R. J., & Owens, S. G. (1991). Cessation from cigarette smoking: Changes in body weight, body composition, resting metabolism, and energy consumption. *Metabolism*, *40*(5), 465–470.

Molnár, D., & Schutz, Y. (1997). The effect of obesity, age, puberty and gender on resting metabolic rate in children and adolescents. *European Journal of Pediatrics*, *156*(5), 376–381.

Moore, F. D. (1980). Energy and the maintenance of the body cell mass. *Journal of Parenteral and Enteral Nutrition*, *4*(3), 228–260.

Moore, L. G., Cymerman, A., Huang, S. Y., McCullough, R. E., McCullough, R. G., Rock, P. B., Young, A., Young, P., Weil, J. V., & Reeves, J. T. (1987). Propranolol blocks metabolic rate increase but not ventilatory acclimatization to 4300 m. *Respiration Physiology*, *70*(2), 195–204.

Morris, C. J., Aeschbach, D., & Scheer, F. A. J. L. (2012). Circadian system, sleep and endocrinology. *Molecular and Cellular Endocrinology*, *349*(1), 91–104.

Morris, C. J., Garcia, J. I., Myers, S., Yang, J. N., Trienekens, N., & Scheer, F. A. J. L. (2015). The human circadian system has a dominating role in causing the morning/evening difference in diet-induced thermogenesis. *Obesity*, *23*(10), 2053–2058.

Mountjoy, M., Ackerman, K. E., Bailey, D. M., Burke, L. M., Constantini, N., Hackney, A. C., Heikura, I. A., Melin, A., Pensgaard, A. M., Stellingwerff, T., Sundgot-Borgen, J. K., Torstveit, M. K., Jacobsen, A. U., Verhagen, E., Budgett, R., Engebretsen, L., & Erdener, U. (2023). 2023 International Olympic Committee's (IOC) consensus statement on Relative Energy Deficiency in Sport (REDs). *British Journal of Sports Medicine*, *57*(17), 1073–1097.

Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R., & Ljungqvist, A. (2014). The IOC consensus statement: Beyond the female athlete triad-relative energy deficiency in sport (RED-S). *British Journal of Sports Medicine*, *48*(7), 491–497.

Mountjoy, M., Sundgot-Borgen, J. K., Burke, L. M., Ackerman, K. E., Blauwet, C., Constantini, N., Lebrun, C., Lundy, B., Melin, A. K., Meyer, N. L., Sherman, R. T., Tenforde, A. S., Torstveit, M. K., & Budgett, R. (2018). IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. *British Journal of Sports Medicine*, *52*(11), 687–697.

Mujika, I., Sharma, A. P., & Stellingwerff, T. (2019). Contemporary periodization of altitude training for elite endurance athletes: A narrative review. *Sports Medicine*, *49*(11), 1651-1669.

Mukundan, H., Kanagy, N. L., & Resta, T. C. (2004). 17-β Estradiol attenuates hypoxic induction of HIF-1α and erythropoietin in Hep3B cells. *Journal of Cardiovascular Pharmacology*, *44*(1), 93–100.

Müller, M. J., & Bosy-Westphal, A. (2013). Adaptive thermogenesis with weight loss in humans. *Obesity*, *21*(2), 218–228.

Müller, M. J., Enderle, J., Pourhassan, M., Braun, W., Eggeling, B., Lagerpusch, M., Glüer, C. C., Kehayias, J. J., Kiosz, D., & Bosy-Westphal, A. (2015). Metabolic adaptation to caloric restriction and subsequent refeeding: The Minnesota Starvation Experiment revisited. *American Journal of Clinical Nutrition*, *102*(4), 807–819.

Müller, M. J., Geisler, C., Hübers, M., Pourhassan, M., Braun, W., & Bosy-Westphal, A. (2018). Normalizing resting energy expenditure across the life course in humans: Challenges and hopes. *European Journal of Clinical Nutrition*, *72*(5), 628–637.

Murray, A. J., & Horscroft, J. A. (2016). Mitochondrial function at extreme high altitude. *Journal of Physiology*, *594*(5), 1137–1149.

Myburgh, K. H., Berman, C., Novick, I., Noakes, T. D., & Lambert, E. V. (1999). Decreased resting metabolic rate in ballet dancers with menstrual irregularity. *International Journal of Sport Nutrition*, *9*(3), 285–294.

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.

Nakayama, T., Suzuki, M., & Ishizuka, N. (1975). Action of progesterone on preoptic thermosensitive neurones. *Nature*, *258*(5530), 80.

Nana, A., Slater, G. J., Hopkins, W. G., & Burke, L. M. (2012). Effects of daily activities on dual-energy X-ray absorptiometry measurements of body composition in active people. *Medicine and Science in Sports and Exercise*, *44*(1), 180–189.

Napolitano, A., Zanin, R., Palma, F., Romani, C., Grandi, G., Di Carlo, C., & Cagnacci, A. (2016). Body composition and resting metabolic rate of perimenopausal women using continuous progestogen contraception. *The European Journal of Contraception & Reproductive Health Care*, *21*(2), 168–175.

Nattiv, A., Agostini, R., Drinkwater, B., & Yeager, K. K. (1994). The female athlete triad. The inter-relatedness of disordered eating, amenorrhea, and osteoporosis. *Clinics in Sports Medicine*, *13*(2), 405–418.

Navalta, J. W., Tanner, E. A., & Bodell, N. G. (2018). Acute normobaric hypoxia exposure and excess post-exercise oxygen consumption. *Aerospace Medicine and Human Performance*, *89*(12), 1031–1035.

Nedeltcheva, A. V., Kilkus, J. M., Imperial, J., Kasza, K., Schoeller, D. A., & Penev, P. D. (2009). Sleep curtailment is accompanied by increased intake of calories from snacks. *American Journal of Clinical Nutrition*, *89*(1), 126–133.

Nehlig, A. (2018). Interindividual differences in caffeine metabolism and factors driving caffeine consumption. *Pharmacological Reviews*, *70*(2), 384–411.

Nishimura, T., Motoi, M., Egashira, Y., Choi, D., Aoyagi, K., & Watanuki, S. (2015). Seasonal variation of non-shivering thermogenesis (NST) during mild cold exposure. *Journal of Physiological Anthropology*, *34*(1), 11.

Nishisaka, M. M., Zorn, S. P., Kristo, A. S., Sikalidis, A. K., & Reaves, S. K. (2022). Assessing dietary nutrient adequacy and the effect of season-long training on body composition and metabolic rate in collegiate male basketball players. *Sports*, *10*(9), 127.

O'Donnell, E., Harvey, P. J., & De Souza, M. J. (2009). Relationships between vascular resistance and energy deficiency, nutritional status and oxidative stress in oestrogen deficient physically active women. *Clinical Endocrinology*, *70*(2), 294–302.

Ong, J. N., Ducker, K. J., Furzer, B. J., Dymock, M., & Landers, G. J. (2022a). Food and fluid intake and hydration status does not affect ultrasound measurements of subcutaneous adipose tissue in active adults. *Journal of Science and Medicine in Sport*, *25*(7), 548–552.

Ong, J. N., Ducker, K. J., Furzer, B. J., Dymock, M., & Landers, G. J. (2022b). 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.

Ormsbee, M. J., & Arciero, P. J. (2012). Detraining increases body fat and weight and decreases VO2peak and metabolic rate. *Journal of Strength and Conditioning Research*, *26*(8), 2087–2095.

Oshima, S., Miyauchi, S., Kawano, H., Ishijima, T., Asaka, M., Taguchi, M., Torii, S., & Higuchi, M. (2012). Fat-free mass can be utilized to assess resting energy expenditure for male athletes of different body size. *Journal of Nutritional Science and Vitaminology*, *57*(6), 394–400.

Osiba, S. (1957). The seasonal variation of basal metabolism and activity of thyroid gland in man. *The Japanese Journal of Physiology*, *7*(4), 355–365.

Osterberg, K. L., & Melby, C. (2000). Effect of acute resistance exercise on postexercise oxygen consumption and resting metabolic rate in young women. *International Journal of Sport Nutrition & Exercise Metabolism*, *10*(1), 71–81.

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.

Oxfeldt, M., Marsi, D., Christensen, P. M., Andersen, O. E., Johansen, F. T., Bangshaab, M., Risikesan, J., Jeppesen, J. S., Hellsten, Y., Phillips, S. M., Ortenblad, N., & Hansen, M. (2024). Low energy availability followed by optimal energy availability does not benefit performance in trained females. *Medicine & Science in Sports & Exercise*, *56*(5), 902–916.

Oxfeldt, M., Phillips, S. M., Andersen, O. E., Johansen, F. T., Bangshaab, M., Risikesan, J., McKendry, J., Melin, A. K., & Hansen, M. (2023). Low energy availability reduces myofibrillar and sarcoplasmic muscle protein synthesis in trained females. *The Journal of Physiology*, *601*(16), 3481–3497.

Özcan, Ö., Den Elzen, W. P. J., Hillebrand, J. J., Den Heijer, M., Van Loendersloot, L. L., Fischer, J., Hamer, H., De Jonge, R., & Heijboer, A. C. (2023). The effect of hormonal contraceptive therapy on clinical laboratory parameters: A literature review. *Clinical Chemistry and Laboratory Medicine*, *62*(1), 18–40.

Pallubinsky, H., Schellen, L., & van Marken Lichtenbelt, W. D. (2019). Exploring the human thermoneutral zone – A dynamic approach. *Journal of Thermal Biology*, *79*, 199–208.

Palmer, B. F., & Clegg, D. J. (2014). Ascent to altitude as a weight loss method: The good and bad of hypoxia inducible factor activation. *Obesity*, *22*(2), 311.

Panissa, V. L. G., Fukuda, D. H., Staibano, V., Marques, M., & Franchini, E. (2021). Magnitude and duration of excess of post-exercise oxygen consumption between high-intensity interval and moderate-intensity continuous exercise: A systematic review. *Obesity Reviews*, *22*(1), 1–16.

Paoli, A., Moro, T., Marcolin, G., Neri, M., Bianco, A., Palma, A., & Grimaldi, K. (2012). High-intensity interval resistance training (HIRT) influences resting energy expenditure and respiratory ratio in non-dieting individuals. *Journal of Translational Medicine*, *10*, 237.

Paolisso, G., Rizzo, M. R., Mazziotti, G., Rotondi, M., Tagliamonte, M. R., Varricchio, G., Carella, C., & Varricchio, M. (2000). Lack of association between changes in plasma

leptin concentration and in food intake during the menstrual cycle. *Journal of Dental Research*, *79*(4), 490–495.

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.

Park, H. K., Yoon, C. A., & Hong, S. K. (1969). Seasonal variations in the basal metabolic rate of the Korean. *Yonsei Medical Journal* , *10*(2), 139–145.

Paschalis, V., Nikolaidis, M. G., Giakas, G., Theodorou, A. A., Sakellariou, G. K., Fatouros, I. G., Koutedakis, Y., & Jamurtas, A. Z. (2010). Beneficial changes in energy expenditure and lipid profile after eccentric exercise in overweight and lean women. *Scandinavian Journal of Medicine and Science in Sports*, *20*(1), 103–111.

Paschalis, V., Nikolaidis, M. G., Theodorou, A. A., Panayiotou, G., Fatouros, I. G., Koutedakis, Y., & Jamurtas, A. Z. (2011). A weekly bout of eccentric exercise is sufficient to induce health-promoting effects. *Medicine and Science in Sports and Exercise*, *43*(1), 64–73.

Patwardhan, R. V., Desmond, P. V., Johnson, R. F., & Schenker, S. (1980). Impaired elimination of caffeine by oral contraceptive steroids. *The Journal of Laboratory and Clinical Medicine*, *95*(4), 603–608.

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. *American Journal of Clinical Nutrition*, *73*(1), 19–26.

Pérez, D. I. V., Soto, D. A. S., Barroso, J. M., dos Santos, D. A., Queiroz, A. C. C., Miarka, B., Brito, C. J., & Quintana, M. S. (2021). Physically active men with high brown adipose tissue activity showed increased energy expenditure after caffeine supplementation. *Journal of Thermal Biology*, *99*, 103000.

Perkins, K. A., Epstein, L. H., Stiller, R. L., Marks, B. L., & Jacob, R. G. (1989). Acute effects of nicotine on resting metabolic rate in cigarette smokers. *American Journal of Clinical Nutrition*, *50*(3), 545-50.

Perkins, K. A., Epstein, L. H., Stiller, R. L., Sexton, J. E., Fernstrom, M. H., Jacob, R. G., & Solberg, R. (1990). Metabolic effects of nicotine after consumption of a meal in smokers and nonsmokers. *American Journal of Clinical Nutrition*, *52*(2), 228–233.

Perkins, K. A., & Sexton, J. E. (1995). Influence of aerobic fitness, activity level, and smoking history on the acute thermic effect of nicotine. *Physiology and Behavior*, *57*(6), 1097–1102.

Perseghin, G., Scifo, P., Pagliato, E., Battezzati, A., Benedini, S., Soldini, L., Testolin, G., Maschio, A. D., & Luzi, L. (2001). Gender factors affect fatty acids-induced insulin resistance in nonobese humans: Effects of oral steroidal contraception. *The Journal of Clinical Endocrinology & Metabolism*, *86*(7), 3188–3196.

Persson, C., Shiel, F., Climstein, M., & Furness, J. (2019). Does a novice technician produce results similar to that of an experienced DXA technician when assessing body composition and bone mineral density? *International Journal of Sport Nutrition and Exercise Metabolism*, *29*(4), 435–440.

Pham, D. D., Lee, J. H., Hong, K. H., Jung, Y. J., Kim, S. J., & Leem, C. H. (2020). Seasonal effects on resting energy expenditure are dependent on age and percent body fat. *Clinical Nutrition*, *39*(4), 1276–1283.

Piers, L. S., Soares, M. J., McCormack, L. M., & O'Dea, K. (1998). Is there evidence for an age-related reduction in metabolic rate? *Journal of Applied Physiology*, *85*(6), 2196–2204.

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.

Plasqui, G., Kester, A. D. M., & Westerterp, K. R. (2003). Seasonal variation in sleeping metabolic rate, thyroid activity, and leptin. *American Journal of Physiology - Endocrinology and Metabolism*, *285*(2), 338–343.

Poehlman, E. T., Despres, J. P., Bessette, H., Fontaine, E., Tremblay, A., & Bouchard, C. (1985). Influence of caffeine on the resting metabolic rate of exercise-trained and inactive subjects. *Medicine and Science in Sports & Exercise*, *17*(6), 689–694.

Poehlman, E. T., Mcauliffe, T. L., Van Houten, D. R., & Danforth, E. (1990). Influence of age and endurance training on metabolic rate and hormones in healthy men. *American Journal of Physiology*, *259*(1 Pt 1), E66-72.

Poehlman, E. T., Melby, C. L., & Badylak, S. F. (1991). Relation of age and physical exercise status on metabolic rate in younger and older healthy men. *Journal of Gerontology: Biological Sciences*, *46*(2), 54–58.

Poehlman, E. T., Toth, M. J., Ades, P. A., & Calles-Escandon, J. (1997). Gender differences in resting metabolic rate and noradrenaline kinetics in older individuals. *European Journal of Clinical Investigation*, *27*(1), 23–28.

Poehlman, E. T., Toth, M. J., & Webb, G. D. (1993). Sodium-potassium pump activity contributes to the age-related decline in resting metabolic rate. *Journal of Clinical Endocrinology and Metabolism* , *76*(4), 1054–1057.

Poehlman, E. T., Melby, C. L., Badylak, S. F. (1988). Resting metabolic rate and postprandial thermogenesis in highly trained and untrained males. *American Journal of Clinical Nutrition*, *47*(1), 793–798.

Pontzer, H., Yamada, Y., Sagayama, H., Ainslie, P. N., Andersen, L. F., Anderson, L. J., Arab, L., Baddou, I., Bedu-addo, K., Blaak, E. E., Lambert, E. V, Leonard, W. R., Lessan, N., Martin, C., & Medin, A. C. (2021). Daily energy expenditure through the human life course. *Metabolism*, *373*(6556), 808–812.

Popp, C. J., Tisch, J. J., Sakarcan, K. E., Bridges, W. C., & Jesch, E. D. (2016). Approximate time to steady-state resting energy expenditure using indirect calorimetry in young, healthy adults. *Frontiers in Nutrition*, *3*, 49.

Purcell, S. A., Johnson-Stoklossa, C., Braga Tibaes, J. R., Frankish, A., Elliott, S. A., Padwal, R., & Prado, C. M. (2020). Accuracy and reliability of a portable indirect calorimeter compared to whole-body indirect calorimetry for measuring resting energy expenditure. *Clinical Nutrition ESPEN*, *39*, 67–73.

Qian, J., Morris, C. J., Caputo, R., Wang, W., Garaulet, M., & Scheer, F. A. J. L. (2019). Sex differences in the circadian misalignment effects on energy regulation. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(47), 23806–23812.

Quatela, A., Callister, R., Patterson, A., & MacDonald-Wicks, L. (2016). The energy content and composition of meals consumed after an overnight fast and their effects on diet induced thermogenesis: A systematic review, meta-analyses and meta-regressions. *Nutrients*, *8*(11), 670.

Ratcliff, L., Gropper, S. S., White, B. D., Shannon, D. M., & Huggins, K. W. (2011). The influence of habitual exercise training and meal form on diet-induced thermogenesis in college-age men. *International Journal of Sport Nutrition and Exercise Metabolism*, *21*(1), 11–18.

Ravussin, E., Schutz, Y., Acheson, K. J., Dusmet, M., Bourquin, L., & Jequier, E. (1985). Short-term, mixed-diet overfeeding in man no evidence for "luxuskonsumption." *American Journal of Physiology - Endocrinology and Metabolism*, *249*(5 Pt 1), E470-7.

Reed, J. L., de Souza, J. J., Mallinson, R. J., Scheid, J. L., & Williams, N. I. (2015). Energy availability discriminates clinical menstrual status in exercising women. *Journal of the International Society of Sports Nutrition*, *12*(1), 1–11.

Reneau, J., Obi, B., Moosreiner, A., & Kidambi, S. (2019). Do we need race-specific resting metabolic rate prediction equations? *Nutrition & Diabetes*, *9*(1), 21.

Rising, R., Whyte, K., Albu, J., & Pi-Sunyer, X. (2015). Evaluation of a new whole room indirect calorimeter specific for measurement of resting metabolic rate. *Nutrition and Metabolism*, *12*(1), 1–9.

Roberts, S. B., Fuss, P., Dallal, G. E., Atkinson, A., Evans, W. J., Joseph, L., Fiatarone, M. A., Greenberg, A. S., & Young, V. R. (1996). Effects of age on energy expenditure and substrate oxidation during experimental overfeeding in healthy men. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *51*(2).

Roberts, S. B., Fuss, P., Heyman, M. B., & Young, V. R. (1995). Influence of age on energy requirements. *American Journal of Clinical Nutrition*, *62*(5 Suppl), 1053S-1058S.

Roberts, S. B., Young, V. R., Fuss, P., Fiatarone, M. A., Richard, B., Rasmussen, H., Wagner, D., Joseph, L., Holehouse, E., & Evans, W. J. (1990). Energy expenditure and subsequent nutrient intakes in overfed young men. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *259*(3), R461-469.

Rodriguez-Sanchez, N., & Galloway, S. D. R. (2015). Errors in dual energy x-ray absorptiometry estimation of body composition induced by hypohydration. *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(1), 60–68.

Roffey, D. M., Byrne, N. M., & Hills, A. P. (2006). Day-to-day variance in measurement of resting metabolic rate using ventilated-hood and mouthpiece & nose-clip indirect calorimetry systems. *Journal of Parenteral and Enteral Nutrition*, *30*(5), 426–432.

Rosenbaum, M., Hirsch, J., Gallagher, D. A., & Leibel, R. L. (2008). Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. *American Journal of Clinical Nutrition*, *88*(4), 906–912.

Rouillier, M. A., David-Riel, S., Brazeau, A. S., St-Pierre, D. H., & Karelis, A. D. (2015). Effect of an acute high carbohydrate diet on body composition using DXA in young men. *Annals of Nutrition and Metabolism*, *66*(4), 233–236.

Rush, E. C., Plank, L. D., & Robinson, S. M. (1997). Resting metabolic rate in young Polynesian and Caucasian women. *International Journal of Obesity*, *21*(11), 1071–1075.

Rynders, C. A., Morton, S. J., Bessesen, D. H., Wright, K. P., & Broussard, J. L. (2020). Circadian rhythm of substrate oxidation and hormonal regulators of energy balance. *Obesity*, *28*(S1), S104–S113.

Sanchez-Delgado, G., Alcantara, J. M. A., Ortiz-Alvarez, L., Xu, H., Martinez-Tellez, B., Labayen, I., & Ruiz, J. R. (2018). Reliability of resting metabolic rate measurements in young adults: Impact of methods for data analysis. *Clinical Nutrition*, *37*(5), 1618–1624.

Sander, M. (2016). Does the sympathetic nervous system adapt to chronic altitude exposure? *Advances in Experimental Medicine and Biology, 903,* 375-393.

Sareban, M., Schiefer, L. M., MacHolz, F., Schäfer, L., Zangl, Q., Inama, F., Reich, B., Mayr, B., Schmidt, P., Hartl, A., Bärtsch, P., Niebauer, J., Treff, G., & Berger, M. M. (2020). Endurance athletes are at increased risk for early acute mountain sickness at 3450 m. *Medicine and Science in Sports and Exercise*, *52*(5), 1109–1115.

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.

Schuenke, M. D., Mikat, R. P., & McBride, J. M. (2002). Effect of an acute period of resistance exercise on excess post-exercise oxygen consumption: Implications for body mass management. *European Journal of Applied Physiology*, *86*(5), 411–417.

Scott, C. B. (1993). Resting metabolic rate variability as influenced by mouthpiece and noseclip practice procedure. *Journal of Burn Care & Rehabilitation*, *14*(5), 573–577.

Segal, K. R. (1987). Comparison of indirect calorimetric measurements of resting energy expenditure with a ventilated hood, face mask, and mouthpiece. *American Journal of Clinical Nutrition*, *45*(6), 1420–1423.

Sharp, T. A., Bell, M. L., Grunwald, G. K., Schmitz, K. H., Sidney, S., Lewis, C. E., Tolan, K., Hill, J. O., Teresa, A., Bell, M. L., Gary, K., Schmitz, K. H., Lewis, C. E., & Tolan, K. I. M. (2002). Differences in resting metabolic rate between White and African-American Young Adults. *Obesity Research*, *10*(8), 726–732.

Shechter, A., Rising, R., Wolfe, S., Albu, J. B., & St-Onge, M. P. (2014). Postprandial thermogenesis and substrate oxidation are unaffected by sleep restriction. *International Journal of Obesity*, *38*(9), 1153–1158.

Short, K. R., & Sedlock, D. A. (1997). Excess postexercise oxygen consumption and recovery rate in trained and untrained subjects. *Journal of Applied Physiology*, *83*(1), 153–159.

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.

Siervo, M., Oggioni, C., Lara, J., Celis-Morales, C., Mathers, J. C., Battezzati, A., Leone, A., Tagliabue, A., Spadafranca, A., & Bertoli, S. (2015). Age-related changes in resting energy expenditure in normal weight, overweight and obese men and women. *Maturitas*, *80*(4), 406–413.

Sim, A., Tan, H. Q., Ali, Y., & Burns, S. F. (2024). Original Investigation: Manipulating energy availability in male endurance runners: a randomised controlled trial. *Applied Physiology, Nutrition, Metabolism*, *49*(9), 1163–1174.

Singhal, V., Lawson, E. A., Ackerman, K. E., Fazeli, P. K., Clarke, H., Lee, H., Eddy, K., Marengi, D. A., Derrico, N. P., Bouxsein, M. L., & Misra, M. (2014). Irisin levels are lower in young amenorrheic athletes compared with eumenorrheic athletes and non-athletes and are associated with bone density and strength estimates. *PLoS ONE*, *9*(6), e100218.

Sjodin, A. M., Forslund, A. H., Westerterp, K. R., Andersson, A. B., Forslund, J. M., & Hambraeus, L. M. (1996). The influence of physical activity on BMR. *Medicine & Science in Sports & Exercise*, *28*(1), 85–91.

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.

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.

Snell, B., Fullmer, S., & Eggett, D. L. (2014). Reading and listening to music increase resting energy expenditure during an indirect calorimetry test. *Journal of the Academy of Nutrition and Dietetics*, *114*(12), 1939–1942.

Soares, M. J., Piers, L. S., O'Dea, K., & Shetty, P. S. (1998). No evidence for an ethnic influence on basal metabolism: an examination of data from India and Australia. *British Journal of Nutrition*, *79*(4), 333–341.

Song, L. L. T., Venkataraman, K., Gluckman, P., Chong, Y. S., Chee, M. W. L., Khoo, C. M., Leow, M. K. S., Lee, Y. S., Tai, E. S., & Khoo, E. Y. H. (2016). Smaller size of high metabolic rate organs explains lower resting energy expenditure in Asian-Indian than Chinese men. *International Journal of Obesity*, *40*(4), 633–638.

Spaeth, A. M., Dinges, D. F., & Goel, N. (2015). Resting metabolic rate varies by race and by sleep duration. *Obesity*, *23*(12), 2349–2356.

Spall, S., & Austin, S. F. (1998). Peer debriefing in qualitative research: Emerging operational models. *Qualitative Inquiry, 4*(2), 280-292.

Speakman, J. R., & Selman, C. (2003). Physical activity and resting metabolic rate. *Proceedings of the Nutrition Society*, *62*(3), 621–634.

Splinter, Z. T., & Wilson, P. (2019). Physiological and perceptual effects of self-selected and classical relaxing music on resting metabolic rate: A crossover trial. *The Journal of Sports Medicine and Physical Fitness*, *59*(7), 1150–1155.

Staal, S., Sjödin, A., Fahrenholtz, I., Bonnesen, K., & Melin, A. K. (2018). Low RMR ratio as a surrogate marker for energy deficiency, the choice of predictive equation vital for correctly identifying male and female ballet dancers at risk. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(4), 412–418.

Stachenfeld, N. S. (2008). Sex hormone effects on body fluid regulation. *Exercise and Sport Sciences Reviews*, *36*(3), 152–159.

Stellingwerff, T., Heikura, I. A., Meeusen, R., Bermon, S., Seiler, S., Mountjoy, M. L., & Burke, L. M. (2021). Overtraining Syndrome (OTS) and Relative Energy Deficiency in Sport (RED-S): Shared pathways, symptoms and complexities. *Sports Medicine*, *51*(11), 2251–2280.

Stellingwerff, T., Mountjoy, M., McCluskey, W. T., Ackerman, K. E., Verhagen, E., & Heikura, I. A. (2023). Review of the scientific rationale, development and validation of the International Olympic Committee Relative Energy Deficiency in Sport Clinical Assessment Tool: V.2 (IOC REDs CAT2)—by a subgroup of the IOC consensus on REDs. *British Journal of Sports Medicine*, *57*(17), 1109–1118.

Stellingwerff, T., Peeling, P., Garvican-Lewis, L. A., Hall, R., Koivisto, A. E., Heikura, I. A., & Burke, L. M. (2019). Nutrition and altitude: Strategies to enhance adaptation, improve performance and maintain health: A narrative review. *Sports Medicine*, *49*(s2), 169–184.

Sterringer, T., & Larson-Meyer, D. E. (2022). RMR ratio as a surrogate marker for low energy availability. *Current Nutrition Reports*, *11*(2), 263–272.

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.

Stewart, C. L., Goody, C. M., & Branson, R. (2005). Comparison of two systems of measuring energy expenditure. *Journal of Parenteral and Enteral Nutrition*, *29*(3), 212–217.

St-Onge, M. P., Roberts, A. L., Chen, J., Kelleman, M., O'Keeffe, M., RoyChoudhury, A., & Jones, P. J. H. (2011). Short sleep duration increases energy intakes but does not change energy expenditure in normal-weight individuals. *American Journal of Clinical Nutrition*, *94*(2), 410–416.

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.

Strock, N. C. A., Koltun, K. J., Southmayd, E. A., Williams, N. I., & De Souza, M. J. (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.

Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Whybrow, S., Horgan, G. W., King, N., & Blundell, J. (2004). Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *286*(2), 350–358.

Ten Haaf, T., & Weijs, P. J. M. (2014). Resting energy expenditure prediction in recreational athletes of 18-35 years: Confirmation of Cunningham equation and an improved weight-based alternative. *PLoS ONE*, *9*(10), e108460.

Terzioglu, M., & Aykut, R. (1954). Variations in basal metabolic rate at 1.85 km altitude. *Journal of Applied Physiology*, *7*(3), 329–332

Thearle, M. S., Pannacciulli, N., Bonfiglio, S., Pacak, K., & Krakoff, J. (2013). Extent and determinants of thermogenic responses to 24 hours of fasting, energy balance, and five different overfeeding diets in humans. *Journal of Clinical Endocrinology and Metabolism*, *98*(7), 2791–2799.

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the Academy of Nutrition and Dietetics*, *116*(3), 501–528.

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.

Thompson, J., & Manore, M. (1996). Predicted and measured resting metabolic rate of male and female endurance athletes. *Journal of the American Dietetic Association*, *96*(1), 30–34.

Thompson, J., Manore, M. M., & Skinner, J. S. (1993). Resting metabolic rate and thermic effect of a meal in low- and adequate-energy intake male endurance athletes. *International Journal of Sport Nutrition*, *3*(2), 194–206.

Tinsley, G. M., Morales, E., Forsse, J. S., & Grandjean, P. W. (2017). Impact of acute dietary manipulations on DXA and BIA body composition estimates. *Medicine and Science in Sports and Exercise*, *49*(4), 823–832.

Toomey, C. M., McCormack, W. G., & Jakeman, P. (2017). The effect of hydration status on the measurement of lean tissue mass by dual-energy X-ray absorptiometry. *European Journal of Applied Physiology*, *117*(3), 567–574.

Tornberg, Å. B., Melin, A., Koivula, F. M., Johansson, A., Skouby, S., Faber, J., & Sjödin, A. (2017). Reduced neuromuscular performance in amenorrheic elite endurance athletes. *Medicine and Science in Sports and Exercise*, *49*(12), 2478–2485.

Torstveit, M. K., Ackerman, K. E., Constantini, N., Holtzman, B., Koehler, K., Mountjoy, M. L., Sundgot-Borgen, J., & Melin, A. (2023). Primary, secondary and tertiary prevention of Relative Energy Deficiency in Sport (REDs): a narrative review by a subgroup of the IOC consensus on REDs. *British Journal of Sports Medicine*, *57*(17), 1119–1126.

Toth, M. J., Gardner, A. W., & Poehlman, E. T. (1995). Training status, resting metabolic rate, and cardiovascular disease risk in middle-aged men. *Metabolism, 44*(3), 340-347.

Tremblay, A., Coveney, S., Despres, J. P., Nadeau, A., & Prud'Homme, D. (1992). Increased resting metabolic rate and lipid oxidation in exercise-trained individuals: Evidence for a role of β-adrenergic stimulation. *Canadian Journal of Physiology and Pharmacology*, *70*(10), 1342–1347.

Tremblay, A., Despres, J. P., Theriault, G., Fournier, G., & Bouchard, C. (1992). Overfeeding and energy expenditure in humans. *The American Journal of Clinical Nutrition*, *56*, 857–862.

Tremblay, A., Nadeau, A., Fournier, G., & Bouchard, C. (1988). Effect of a three-day interruption of exercise-training on resting metabolic rate and glucose-induced thermogenesis in training individuals. *International Journal of Obesity*, *12*(2), 163–168.

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.

Turley, K. R., McBride, P. J., & Wilmore, J. H. (1993). Resting metabolic rate measured after subjects spent the night at home vs at a clinic. *American Journal of Clinical Nutrition*, *58*(2), 141–144.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M. A. F. L., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., & Teule, G. J. J. (2009). Cold-activated brown adipose tissue in healthy men. *New England Journal of Medicine*, *360*(15), 1500–1508.

Van Ooijen, A. M. J., Van Marken Lichtenbelt, W. D., Van Steenhoven, A. A., & Westerterp, K. R. (2004). Seasonal changes in metabolic and temperature responses to cold air in humans. *Physiology & Behavior*, *82*(2–3), 545–553.

Van Pelt, R. E., Jones, P. P., Davy, K. P., Desouza, C. A., Tanaka, H., Davy, B. M., & Seals, D. R. (1997). Regular exercise and the age-related decline in resting metabolic rate in women. *Journal of Clinical Endocrinology and Metabolism*, *82*(10), 3208–3212.

Vaughan, L., Zurlo, F., & Ravussin, E. (1991). Aging and energy expenditure. *American Journal of Clinical Nutrition*, *53*, 821–825.

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.

Vinales, K. L., Schlögl, M., Piaggi, P., Hohenadel, M., Graham, A., Bonfiglio, S., Krakoff, J., & Thearle, M. S. (2017). The consistency in macronutrient oxidation and the role for epinephrine in the response to fasting and overfeeding. *Journal of Clinical Endocrinology and Metabolism*, *102*(1), 279–289.

Visser, M., Deurenberg, P., Van Staveren, W. A., & Hautvast, J. G. (1995). Resting metabolic rate and diet-induced thermogenesis in young and elderly subjects: relationship with body composition, fat distribution, and physical activity level. *American Journal of Clinical Nutrition*, *61*, 772–780.

Wahrlich, V., Anjos, L. A., Going, S. B., & Lohman, T. G. (2006). Validation of the VO2000 calorimeter for measuring resting metabolic rate. *Clinical Nutrition*, *25*(4), 687–692.

Walker, J., Collins, L. C., Nannini, L., & Stamford, B. A. (1992). Potentiating effects of cigarette smoking and moderate exercise on the thermic effect of a meal. *International Journal of Obesity*, *16*(5), 341–347.

Walker, J. F., & Kane, C. J. (2002). Effects of body mass on nicotine-induced thermogenesis and catecholamine release in male smokers. *Acta Physiologica Sinica*, *54*(4), 405–410.

Wang, X., Wang, Y., Ma, Z., Xu, Y., & Wu, Q. (2017). Indirect calorimetry using a ventilated hood may be easier than using a facemask to achieve steady state when measuring resting energy expenditure. *Nutrition Research*, *48*, 33–39.

Wang, Z. (2012). High ratio of resting energy expenditure to body mass in childhood and adolescence: A mechanistic model. *American Journal of Human Biology*, *24*(4), 460–467.

Wang, Z., Heshka, S., Gallagher, D., Boozer, C. N., Kotler, D. P., & Heymsfield, S. B. (2000). Resting energy expenditure-fat-free mass relationship: New insights provided

by body composition modeling. *American Journal of Physiology - Endocrinology and Metabolism*, *279*(3), 539–545.

Wang, Z., Heshka, S., Heymsfield, S. B., Shen, W., & Gallagher, D. (2005). A cellular-level approach to predicting resting energy expenditure across the adult years. *American Journal of Clinical Nutrition*, *81*, 799–806.

Wang, Z. M., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., Later, W., Heymsfield, S. B., & Müller, M. J. (2010). Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure. *The American Journal of Clinical Nutrition*, *92*(6), 1369.

Warwich, P. M., Chapple, R. S., & Thomson, E. S. (1987). The effect of smoking two cigarettes on resting metabolic rate with and without food. *International Journal of Obesity*, *11*(3), 229–237.

Warwick, P. M., Edmundson, H. M., & Thomson, E. S. (1995). No evidence for a chronic effect of smoking on energy expenditure. *International Journal of Obesity and Related Metabolic Disorders*, *19*(3), 198–201.

Wefers, J., Van Moorsel, D., Hansen, J., Connell, N. J., Havekes, B., Hoeks, J., Van Marken Lichtenbelt, W. D., Duez, H., Phielix, E., Kalsbeek, A., Boekschoten, M. V., Hooiveld, G. J., Hesselink, M. K. C., Kersten, S., Staels, B., Scheer, F. A. J. L., & Schrauwen, P. (2018). Circadian misalignment induces fatty acid metabolism gene profiles and compromises insulin sensitivity in human skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(30), 7789–7794.

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.

Weir, J. B. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology*, *109*(1–2), 1–9.

Welch, W. A., Strath, S. J., & Swartz, A. M. (2015). Congruent validity and reliability of two metabolic systems to measure resting metabolic rate. *International Journal of Sports Medicine*, *36*(5), 414–418.

Wells, J. C. K. (2007). Sexual dimorphism of body composition. *Best Practice and Research: Clinical Endocrinology and Metabolism*, *21*(3), 415–430.

Westerterp, K. R. (2003). Energy metabolism and body composition: general principles. *European Respiratory Monograph*, *24*, 1–10.

Westerterp, K. R. (2013). Metabolic adaptations to over - and underfeeding - Still a matter of debate? *European Journal of Clinical Nutrition*, *67*(5), 443–445.

Westerterp, K. R., Meijer, G. A. L., Schoffelen, P., & Janssen, E. M. E. (1994). Body mass, body composition and sleeping metabolic rate before, during and after endurance exercise. *European Journal of Applied Physiology*, *69*, 203–208.

Weststrate, J. A., & Hautvast, J. G. A. J. (1990). The effects of short-term carbohydrate overfeeding and prior exercise on resting metabolic rate and diet-induced thermogenesis. *Metabolism*, *39*(12), 1232–1239.

Weststrate, J. A., Weys, P. J. M., Poortvliet, E. J., Deurenberg, P., & Hautvast, J. (1989). Diurnal variation in postabsorptive resting metabolic rate and diet-induced thermogenesis. *American Journal of Clinical Nutrition*, *50*(5), 908–914.

Willis, J. W. (2007). *Foundations of qualitative research: Interpretive and critical approaches frameworks for qualitative research*. SAGE Publications Inc.

Wilmore, J. H., Stanforth, P. R., Hudspeth, L. A., Gagnon, J., Daw, E. W., Leon, A. S., Rao, D. C., Skinner, J. S., & Bouchard, C. (1998). Alterations in resting metabolic rate as a consequence of 20 wk of endurance training: The HERITAGE Family Study. *American Journal of Clinical Nutrition*, *68*(1), 66–71.

Woods, A. L., Garvican-Lewis, L. A., Rice, A. J., & Thompson, K. G. (2016). The ventilation-corrected ParvoMedics TrueOne 2400 provides a valid and reliable assessment of resting metablic rate (RMR) in athletes compared with the Douglas Bag method. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(5), 454–463.

Woods, A. L., Garvican-Lewis, L. A., Rice, A., & Thompson, K. G. (2017). 12 days of altitude exposure at 1800 m does not increase resting metabolic rate in elite rowers. *Applied Physiology, Nutrition and Metabolism*, *42*(6), 672–676.

Woods, A. L., Rice, A. J., Garvican-Lewis, L. A., Wallett, A. M., Lundy, B., Rogers, M. A., Welvaert, M., Halson, S., McKune, A., & Thompson, K. G. (2018). The effects of intensified training on resting metabolic rate (RMR), body composition and performance in trained cyclists. *PLoS ONE*, *13*(2), 1–25.

Woods, A. L., Sharma, A. P., Garvican-Lewis, L. A., Saunders, P. U., Rice, A. J., & Thompson, K. G. (2017). Four weeks of classical altitude training increases resting metabolic rate in highly trained middle-distance runners. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(1), 83–90.

Wouters-Adriaens, M. P. E., & Westerterp, K. R. (2008). Low resting energy expenditure in Asians can be attributed to body composition. *Obesity*, *16*(10), 2212–2216.

Yeager, K. K., Acostini, R., Nattiv, A., & Drinkwater, B. (1993). The female athletic triad: Disordered eating, amenorrhea, and osteoporosis. *Medicine and Science in Sports and Exercise*, *25*(7), 775–777.

Yeung, S. S. Y., Trappenburg, M. C., Meskers, C. G. M., Maier, A. B., & Reijnierse, E. M. (2020). The use of a portable metabolic monitoring device for measuring RMR in healthy adults. *The British Journal of Nutrition*, *124*(11), 1229.

Young, A. J., Margolis, L. M., & Pasiakos, S. M. (2019). Commentary on the effects of hypoxia on energy substrate use during exercise. *Journal of the International Society of Sports Nutrition*, *16*(1), 28.

Zabriskie, H. A., Currier, B. S., Harty, P. S., Stecker, R. A., Jagim, A. R., & Kerksick, C. M. (2019). Energy status and body composition across a collegiate women's lacrosse season. *Nutrients*, *11*(2).

Zhang, S., Osumi, H., Uchizawa, A., Hamada, H., Park, I., Suzuki, Y., Tanaka, Y., Ishihara, A., Yajima, K., Seol, J., Satoh, M., Omi, N., & Tokuyama, K. (2020). Changes in sleeping energy metabolism and thermoregulation during menstrual cycle. *Physiological Reports*, *8*(2).

Zheng, X. M., & Williams, R. C. (2002). Serum caffeine levels after 24-hour abstention: Clinical implications on dipyridamole 201T1 myocardial perfusion imaging. *Journal of Nuclear Medicine Technology*, *30*(3), 123–127.

Zitting, K. M., Vujovic, N., Yuan, R. K., Isherwood, C. M., Medina, J. E., Wang, W., Buxton, O. M., Williams, J. S., Czeisler, C. A., & Duffy, J. F. (2018). Human resting energy expenditure varies with circadian phase. *Current Biology*, *28*(22), 3685.

Zouhal, H., Jacob, C., Delamarche, P., & Gratas-Delamarche, A. (2008). Catecholamines and the effects of exercise, training and gender. *Sports Medicine*, *38*(5), 401–423.

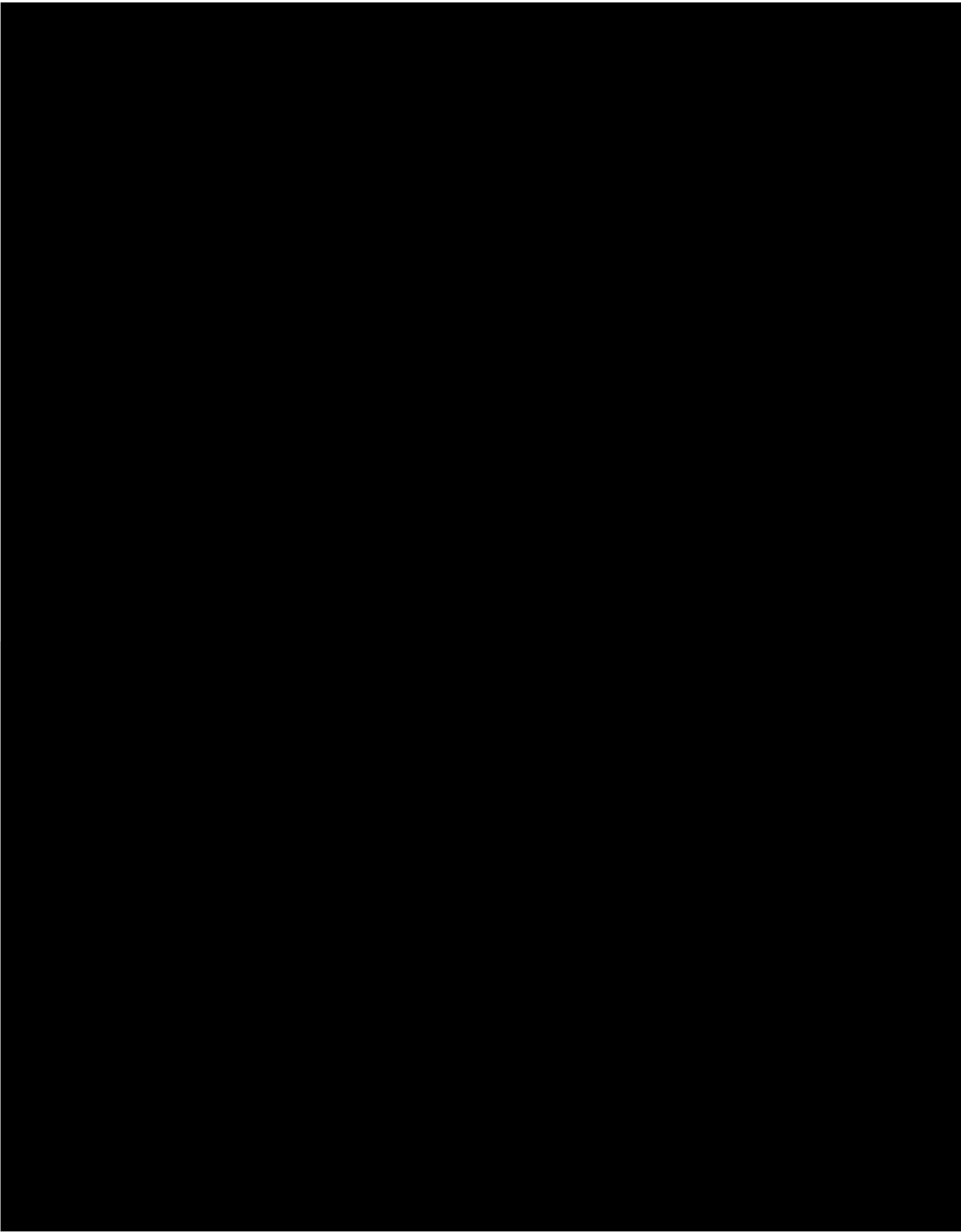
Zurlo, F., Schutz, Y., Frascarolo, P., Enzi, G., Deriaz, O., & Jequier, E. (1986). Variability of resting energy expenditure in healthy volunteers during fasting and continuous enteral feeding. *Critical Care Medicine*, *14*(6), 536–538.

11 Research Portfolio Appendix

11.1 Best Practice Guidelines for RMR Measurements in Australian High Performance Sport System

[redacted]

11.2 Australian Institute of Sport Insight Report on RMR measurements



11.3 Publication statements of contribution of others

1. **Kuikman, M.A.**, McKay, A.K.A., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Smith, E.S., McCormick, R., Tee, N., Minahan, C., Skinner, J., Ackerman, K.E., and Burke, L.M. (2024). Effect of menstrual cycle phase and hormonal contraceptives on resting metabolic rate and body composition. *International Journal of Sport Nutrition and Exercise Metabolism, 34*(4),207-217. doi: 10.1123/ijsnem.2023-0193.

Contribution statement: MK was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, and drafting, revising and approval of final manuscript. AM contributed to the conception and design, collection and assembly of data, data analysis and interpretation, and revising and approval of final manuscript. RH contributed to the conception and design, data interpretation and revising and approval of final manuscript. KES contributed to the data interpretation and revising and approval of final manuscript. TS contributed to the data interpretation and revising and approval of final manuscript. ES contributed to the conception and design, collection and assembly of data, and revising and approval of final manuscript. RM contributed to the collection and assembly of data, and revising and approval of final manuscript. NT contributed to the collection and assembly of data, and revising and approval of final manuscript. CM contributed to the conception and design, data interpretation and revising and approval of final manuscript. JS contributed to the conception and design and revising and approval of final manuscript. KA contributed to the data interpretation and revising and approval of final manuscript. LB contributed to the conception and design, collection and assembly of data, data interpretation, and revising and approval of final manuscript.

Approximate percentage contributions: M.A. Kuikman 60%; A.K.A. McKay 6.5%; R. Harris 3%; K.J. Elliott-Sale 3%; T. Stellingwerff 3%; E.S. Smith 3%; R. McCormick 3%; N. Tee 3%; C. Minahan 3%; J. Skinner 3%; K.E. Ackerman 3%; L.M. Burke 6.5%.

I acknowledge that my contribution to the above paper is 60%

M.A. Kuikman Date: 18/10/2024

As principal supervisor of this project, I certify that the above contributions are true and correct:

L.M. Burke Date: 23/10/2024

Coauthor signatures:

A.K.A. McKay Date: 18/10/2024

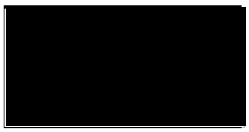
R. Harris Date: 24/10/2024

K.J. Elliott-Sale Date: 6/11/2024

T. Stellingwerff Date: 18/10/24

E.S. Smith Date: 18/10/2024

R. McCormick Date: 18/10/2024



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2. **Kuikman, M.A.,** McKay, A.K.A., McCormick, R., Tee, N., Vallance, B., Ackerman, K.E. Harris, R., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. The temporal effects of altitude and low energy availability manipulation on resting metabolic rate in female athletes. *Medicine & Science in Sports & Exercise.* 57(1), 123-133. doi: 10.1249/MSS.0000000000003534.

Contribution statement: MK was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, and drafting, revising and approval of final manuscript. AM contributed to the conception and design, collection and assembly of data, data analysis and interpretation, and revising and approval of final manuscript. RM contributed to the conception and design, collection and assembly of data, and revising and approval of final manuscript. NT contributed to the collection and assembly of data, and revising and approval of final manuscript. BV contributed to the conception and design, collection and assembly of data, and revising and approval of final manuscript. KA contributed to the data interpretation and revising and approval of final manuscript. RH contributed to the data interpretation and revising and approval of final manuscript. KES contributed to the data interpretation and revising and approval of final manuscript. TS contributed to the data interpretation and revising and approval of final manuscript. LB contributed to the conception and design, collection and assembly of data, data interpretation, and revising and approval of final manuscript.

Approximate percentage contributions: M.A. Kuikman 60%; A.K.A. McKay 7.5%; R. McCormick 5%; N. Tee 5%; B. Vallance 5%; K.E. Ackerman 2.5%; R. Harris 2.5%; K.J. Elliott-Salte 2.5%; T. Stellingwerff 2.5%; L.M. Burke 7.5%.

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K.J. Elliott-Sale Date: 6/11/2024

T. Stellingwerff Date: 18/10/2024

3. **Kuikman, M.A.,** Smith, E., McKay, A.K.A., McCormick, R., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. (2024). Impact of acute dietary and exercise manipulation on next day RMR measurements and DXA body composition estimates. *Medicine & Science in Sports & Exercise*. doi: 10.1249/MSS.0000000000003555. [Online ahead of print]

Contribution statement: MK was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, and drafting, revising and approval of final manuscript. ES contributed to the conception and design, collection and assembly of data, and revising and approval of final manuscript. AM contributed to the conception and design, collection and assembly of data, data interpretation, and revising and approval of final manuscript. RM contributed to the collection and assembly of data, and revising and approval of final manuscript. KA contributed to the data interpretation and revising and approval of final manuscript. RH contributed to the data interpretation and revising and approval of final manuscript. KES contributed to the data interpretation and revising and approval of final manuscript. TS contributed to the data interpretation and revising and approval of final manuscript. LB contributed to the conception and design, collection and assembly of data, data interpretation, and revising and approval of final manuscript.

Approximate percentage contributions: M.A. Kuikman 60%; E. Smith 10%; A.K.A. McKay 7.5%; R. McCormick 5%; K.E. Ackerman 2.5%; R. Harris 2.5%; K.J. Elliott-Sale 2.5%; T. Stellingwerff 2.5%; L.M. Burke 7.5%.

I acknowledge that my contribution to the above paper is 60%

M.A. Kuikman Date: 18/10/2024

As principal supervisor of this project, I certify that the above contributions are true and correct:

L.M. Burke Date: 23/10/2024

Coauthor signatures:

E. Smith Date: 18/10/2024

A.K.A. McKay Date: 18/10/2024

R. McCormick Date: 18/10/2024

K.E. Ackerman Date: 17/10/24

R. Harris Date: 24/10/2024

K.J. Elliott-Sale Date: 6/11/2024

T. Stellingwerff Date: 18/10/2024

4. **Kuikman, M.A.,** McKay, A.K.A., Brown, H., Townsend, N., McCormick, R., Morabito, A., Pichshev, N., Slater, G., and Burke, L.M. (2024). Barriers and enablers to measuring resting metabolic rate in the high-performance sporting system: A qualitative exploratory study. Currently in review with Journal of Sports Science.

Contribution statement: MK was primarily responsible for the conception and design, collection and assembly of data, data analysis and interpretation, and drafting, revising and approval of final manuscript. AM contributed to the revising and approval of final manuscript. HB contributed to the data analysis and interpretation and revising and approval of final manuscript. NT contributed to the conception and design, and revising and approval of final manuscript. RM contributed to the conception and design, and revising and approval of final manuscript. AM contributed to the conception and design, and revising and approval of final manuscript. NP contributed to the conception and design, and revising and approval of final manuscript. GS contributed to the conception and design, and revising and approval of final manuscript. LBM contributed to the conception and design, and revising and approval of final manuscript.

Approximate percentage contributions: M.A. Kuikman 60%; A.K.A. McKay 2.5%; H. Brown 16.5%; N. Townsend 3.5%; R. McCormick 3.5%; A. Morabito 3.5%; N. Pichshev 3.5%; G. Slater 3.5%; L.M. Burke 3.5%.

I acknowledge that my contribution to the above paper is 60%

M.A. Kuikman Date: 18/10/2024

As principal supervisor of this project, I certify that the above contributions are true and correct:

L.M. Burke Date: 23/10/2024

Coauthor signatures:

A.K.A. McKay Date: 18/10/2024

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11.4 Conference statements of contribution of others

1. Kuikman, M.A., McKay, A.K.A., McCormick, R., Tee, N., Vallance, B., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. The temporal effects of altitude and low energy availability manipulation on resting metabolic rate in female athletes.

Abstract for oral presentation at the 2024 Women in Sport Congress, Sydney, Australia, March 2024.

Contribution statement: This presentation was based on the work from study 2 (author contribution listed in section 11.3) and was created and delivered by MK.

2. Kuikman, M.A., McKay, A.K.A., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., Smith, E.S., McCormick, R., Tee, N., Minahan, C., Skinner, J., Ackerman, K.E. and Burke, L.M. Effect of menstrual cycle phase and hormonal contraceptives on resting metabolic rate and body composition.

Abstract for poster presentation at 2023 American College of Sports Medicine Annual Meeting, Colorado, America, June 2023.

Contribution statement: This presentation was based on the work from study 1 (author contribution listed in section 11.3) and was created and delivered by MK and reviewed by all authors.

3. Kuikman, M.A., Smith, E.S., McKay, A.K.A., Ackerman, K.E., Harris, R., Elliott-Sale, K.J., Stellingwerff, T., and Burke, L.M. Fuelling the female athlete: auditing her representation in studies of acute carbohydrate intake for exercise.

Abstract for podium presentation at the 2022 Women in Sport Congress, Melbourne, Australia, August 2022.

Contribution statement: This presentation was based on the work from a study not part of this thesis and was created and delivered by MK.

11.5 Ethics approval for studies 1-4

Australian Catholic University
Human Research Ethics Committee
Project Approval Certificate

Chief Investigator(s)/Supervisor(s):	Professor Louise Burke
Co-Investigator(s):	
Student Researcher(s):	Megan Kuikman
Project title:	The Female Athlete Research Camp: Exploring interactions between performance, health, and the menstrual cycle
Project approval date:	21/01/2022
Project approval end date:	22/08/2024
Human Research Ethics Committee (HREC) Register Number:	**2021-285**HI
Chief Investigator(s)/Supervisor(s):	Professor Louise Burke
Co-Investigator(s):	Megan Kuikman
Student Researcher(s):	
Project title:	The Effects of Acute Manipulation of Diet and Exercise on Resting Metabolic Rate Variability, Performance and Metabolism
Project approval date:	26/05/2022
Project approval end date:	31/05/2023
Human Research Ethics Committee (HREC) Register Number:	**2022-2561**H
Chief Investigator(s)/Supervisor(s):	Professor Louise Burke
Co-Investigator(s):	
Student Researcher(s):	Megan Kuikman
Project title:	Effects of physique manipulation via an LEA diet on haematological adaptations to altitude training.
Project approval date:	29/08/2022
Project approval end date:	31/08/2023
Human Research Ethics Committee (HREC) Register Number:	**2022-2701**HC
Chief Investigator(s)/Supervisor(s):	Professor Louise Burke
Co-Investigator(s):	
Student Researcher(s):	Megan Kuikman
Project title:	Measurement of Resting Metabolic Rate in Athletes: An Insight Report
Project approval date:	13/12/2023
Project approval end date:	02/01/2025
Human Research Ethics Committee (HREC) Register Number:	**2023-3380**E

This is to certify that the above applications have been reviewed by the Australian Catholic University Human Research Ethics Committee (ACU HREC). The applications were approved for the period given above.

Continued approval of research projects is contingent upon the submission of an annual progress report which is due on/before each anniversary of the project approval. A final report is due upon completion of the project. A report proforma can be downloaded from the website (link below).

Researchers are responsible for ensuring that all conditions of approval are adhered to and that any modifications to the protocol, including changes to personnel, are approved prior to implementation. In addition, the ACU HREC must be notified of any reportable matters including, but not limited to, incidents, complaints and unexpected issues.

Researchers are also responsible for ensuring that they adhere to the requirements of the *National Statement on Ethical Conduct in Human Research,* the *Australian Code for the Responsible Conduct of Research* and the University's *Research Code of Conduct*.

Any queries relating to this application should be directed to the Research Ethics and Integrity Office (Res.Ethics@acu.edu.au).

Kind regards,

Leanne Stirling

Research Ethics & Integrity Officer
On behalf of the ACU HREC Chair, Associate Professor Erin Conway

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