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The effect of reproductive hormones and energy availability on muscle function, cognition and bone metabolism in females

By

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Copyright statement

Abstract

The Relative Energy Deficiency in Sport (RED-S) model has proposed that several health and performance factors, including muscle strength and elements of cognitive function, are affected by reduced energy availability (EA) in athletes, yet there is currently limited evidence available to support this. Furthermore, it is not known whether alterations to reproductive hormones, that occur with low EA, impact these factors in the same way that bone metabolism is synergistically affected by hypothalamic amenorrhea and low EA in the Female Athlete Triad. Any potential effects of reproductive hormones are further confounded by hormonal contraceptive (HC) use, which down-regulates reproductive hormones, while supplying exogenous reproductive hormones to the system, although the prevalence of HC use in female athletes is not known.

In Chapter 4, it was shown that quadriceps and first dorsal interosseus (FDI) muscle force measurements were reliable (ICC = 0.990-002, CV = 3.21-3.22 %) and suitable for use in subsequent chapters (Chapters 6 and 9).

In Chapter 5, it was shown that approximately half (49.5%) of 430 elite female athletes used HCs, with 50.5% using no form of HC, evidencing the importance of considering both HC users and non-users in future research. The majority (69.8%) of HC use was comprised of combined oral contraceptives (OCs) and the main reason for using these preparations was the perceived ability to manipulate menstruation.

In Chapters 6-8, muscle force production (quadriceps force, FDI muscle force and countermovement jump height), cognitive function (verbal memory, spatial awareness, verbal fluency, psychomotor performance, executive inhibition and attention) and bone metabolism (Carboxy-terminal cross-linking telopeptide of type 1 collagen [β-CTX], Procollagen Type 1 N Propeptide [P1NP] and bone-specific alkaline phosphatase [BAP]) were assessed across the menstrual cycle (early follicular [EF], ovulatory [OV] and mid-luteal [ML] phases; n= 14) and OC cycle (early pill consumption [PC1], late pill consumption [PC2] and pill-free interval [PFI]; n=14). Muscle force production, cognitive function and bone formation (P1NP, BAP) were not affected by menstrual cycle or OC phase (main effect time; all $P > 0.05$). This was despite significant variations in oestrogen concentrations between menstrual cycle phases (main effect time; $P < 0.05$) and measuring during the pill-free interval (PFI) and early and late pill consumption in OC users. Bone resorption marker (β-CTX) concentrations, however, were significantly higher (+16.0%; $P = 0.014$; $d = 0.37$) during PC1 compared to PC2, showing the importance of exogenous hormones in regulating bone metabolism.

In Chapters 9-10, 20 participants (10 eumenorrheic, 10 OC users) completed three, 3-day conditions: controlled-balanced EA without exercise (BAL; 45 kcal·kg·LBM^{-1}·day^{-1}), diet-induced low EA without exercise (DIET; 15 kcal·kg·LBM^{-1}·day^{-1}) and exercise-induced low EA (EX; 15 kcal·kg·LBM^{-1}·day^{-1}, including 30 kcal·kg·LBM^{-1}·day^{-1} treadmill running at 70% $\dot{V}O_2$max). Muscle force (quadriceps and FDI muscle) and cognitive function (verbal memory, spatial awareness, psychomotor performance, executive inhibition and attention) were measured before and after each condition. DIET had no effect on muscle force production or cognitive function, while EX significantly impaired quadriceps force production (-13.2%; P < 0.05) and mental rotation accuracy (-1.4%; P < 0.05). The muscle and cognitive response to low EA were not different between eumenorrheic women and OC users (all P > 0.05).

In conclusion, changes to the reproductive hormone environment that occur with low EA are not as important a consideration for muscle function and cognitive function compared to bone metabolism. Furthermore, short-term low EA achieved via dietary restriction does not affect muscle force production or cognitive function, but exercise-induced low EA impairs muscle force production and aspects of cognition, evidencing the importance of considering the method by which low energy is achieved in the RED-S model.

List of abbreviations

β-CTX - Carboxy-terminal cross-linking telopeptide of type 1 collagen

ANOVA – Analysis of Variance

BAL – Controlled-balanced Energy Availability

BAP – Bone-specific Alkaline Phosphatase

BMI – Body Mass Index

BMD – Bone Mineral Density

BRUMS – Brunel Mood Scale

Calpain – Calcium-activated cysteine protease

CMJA – Countermovement Jump with Arm Swing

CV – Coefficient of Variation

CVLT – California Verbal Learning Test

DEI – Dietary Energy Intake

DIET – Diet-induced energy restriction

DNS – Dose not specified

DPD - Deoxypyridoline

DXA – Dual-energy X-ray Absorptiometry

E2 – Oestradiol

E3G – Oestrone 3 Gluconaride

EA – Energy Availability

ECLIA – Electrochemiluminescence immunoassay

EDTA – Ethylenediaminetetraacetic Acid

EEE – Exercise Energy Expenditure

EF – Early Follicular

EL – Early Luteal

ELISA – Enzyme-linked immunosorbent assay

EO – Ethinyl Oestradiol

ER – Oestrogen Receptor

EU – Eumenorrheic

EX – Exercise-induced energy restriction

FDI – First Dorsal Interosseus

FP – Follicular Phase

FSH – Follicle Stimulating Hormone

GABA – Gamma-aminobutyric acid

GH – Growth hormone

GnRH – Gonadotrophin-releasing hormone

GPER1 – G Protein-coupled Estrogen Receptor 1

HC – Hormonal Contraceptive

HRT – Hormone Replacement Therapy

HPA – Hypothalamic-pituitary-adrenal

Hpy - Hydroxyproline

ICC – Intra-class Correlation

ICTP – Carboxy-terminal Telopeptide of Type-1 Collagen

IFCC – International Federation of Clinical Chemistry and Laboratory Medicine

IL-6 – Interleukin 6

IL-6r – Interleukin-6 Receptor

IGF-1 – Insulin-like Growth Factor 1

IOF – International Osteoporosis Foundation

IPAQ – International Physical Activity Questionnaire

ISAK – International Society for the Advancement of Kinanthropometry

IUD – Intra-uterine Device

IUS – Intra-uterine System

IVF – In-vitro Fertilisation

LBM – Lean Body Mass

LF – Late Follicular

LH - Luteinizing Hormone

LL – Late Luteal

LNG – Levonorgestrel

LoA –Limits of Agreement

LPD – Luteal Phase Defect

MET – Metabolic Equivalent

ML – Mid Luteal Phase

MVIF – Maximal Voluntary Isometric Force

N.S. – Not specified

NTX – N-terminal telopeptide

OC – Oral Contraceptive

OPG – Osteoprotegerin

OV – Ovulatory Phase

OVS – Ovarian Supressed

P1NP – Procollagen Type 1 N Propeptide

P1CP – Procollagen Type 1 C Propeptide

P_4 – Progesterone

PC1 – Pill consumption 1

PC2 – Pill consumption 2

PCOS – Polycystic Ovarian Syndrome

PdG – Pregnandiol Gluconaride

PFI – Pill Free Interval

PMS – Pre-menstrual syndrome

POMS – Profile of Mood States

PR – Progesterone Receptor

PSQI – Pittsburgh Sleep Quality Index

PTH – Parathyroid Hormone

PYD – Pyridinoline

RANKL - Receptor-activator of nuclear factor kappa-B ligand

RAVLT – Rey Auditory Verbal Learning Test

RED-S – Relative Energy Deficiency in Sport

REE – Resting Energy Expenditure

RVIP – Rapid Visual Information Processing

SCOFF – Sick, Control, One stone, Fat, Food

SHBG – Sex Hormone-binding Globulin

SMD – Sub-clinical Menstrual Disturbances

T_3 – Triiodothyronine

T – Testosterone

TMD – Total Mood Disturbance

Triad – Female Athlete Triad

$\dot{V}O_2max$ – Maximal Oxygen Uptake

$\dot{V}O_2peak$ – Peak oxygen uptake

Glossary of terms

Balanced energy availability – Energy availability > 45 kcal·kgLBM^{-1}·day^{-1}

Low energy availability – Energy availability < 30 kcal·kgLBM^{-1}·day^{-1}

Reduced energy availability - Energy availability > 30 and < 45 kcal·kgLBM^{-1}·day^{-1}

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Acknowledgements

Table of contents

List of tables

List of plates

List of figures

Chapter 1.0. General introduction

The female reproductive system regulates the production of the primary ovarian steroid hormones oestrogen and progesterone, which have the potential to affect athletes' health and performance (Janse de Jonge, 2003; Martin & Elliott-Sale, 2016). In women of reproductive age, the menstrual cycle results in approximately monthly fluctuations in these reproductive hormones, although this process can be disrupted by 'stressors' such as an energy deficiency, disease states and psychological stress (Redman & Loucks, 2005), resulting in a down-regulation of these reproductive hormones. Alterations to the reproductive axis may have physiological consequences in females, so it is important to consider how changes to the reproductive hormone environment affects physiological function.

The down-regulation of reproductive hormone concentrations, termed hypothalamic amenorrhea, occurs frequently in athletic or exercising female populations (Gibbs, Williams, & De Souza, 2013; Reed, De Souza, Mallinson, Scheid, & Williams, 2015). This is a result of the limited energy available to maintain physiological function due to either restricting dietary energy intake, expending excessive amounts of energy in exercise, or a combination of these practices (De Souza et al., 2014; Loucks, Kiens, & Wright, 2011). In addition to reduced oestrogen and progesterone concentrations, low energy availability results in other hypometabolic, changes such as reduced resting energy expenditure (REE), increased ghrelin and reduced leptin and triiodothyronine (T_3) concentrations (Ihle & Loucks, 2004; Martin et al., 2007; Redman & Ravussin, 2009). The combination of low energy availability and menstrual dysfunction, has been shown to negatively impact bone health, with this relationship termed the Female Athlete Triad (Triad; De Souza et al., 2014; Nattiv et al., 2007).

In a recent consensus statement by the International Olympic Committee (Mountjoy et al., 2014), it was suggested that the negative effects of low energy availability may not be limited to impaired menstrual function and bone health. The Relative Energy Deficiency in Sport (RED-S) model suggests that low energy availability may also affect other aspects of physiological function, including the immune system, growth and development, training responses, muscle strength and components of cognitive function (Mountjoy et al., 2014). It has, however, been debated whether these additional aspects should be included (De Souza et al., 2014; Mountjoy et al., 2015) given that many of the proposed effects of low energy availability in the RED-S model are based upon anecdotal evidence from clinical observations (De Souza et al., 2014). This stands in contrast to the Triad, where there is more direct evidence of the interrelationships between low energy availability, menstrual dysfunction and reduced bone health (De Souza et al., 2014). Advocates of both the Triad and RED-S models suggest that further research is required to assess whether the aspects of physiological function

proposed in the RED-S model are indeed influenced by low energy availability (De Souza et al., 2014; Mountjoy et al., 2015).

In the Triad, low energy availability and menstrual dysfunction can act synergistically to impair bone health (Nattiv et al., 2007), although it is not yet clear if low energy availability and menstrual dysfunction interact to affect the other factors identified in the RED-S model. This may be particularly important for aspects of physiological function that are known to be affected by reduced reproductive hormone concentrations. The menopause, which results in reproductive hormone concentrations similar to hypothalamic amenorrhea (Meczekalski, Podfigurna-Stopa, Warenik-Szymankiewicz, & Genazzani, 2008), has been shown to accelerate the onset of sarcopenia (Messier et al., 2011), the cognitive decline (Farrag, Khedr, Abdel-Aleem, & Rageh, 2002) and osteoporosis (Finkelstein et al., 2008), which can be ameliorated with hormone replacement therapy (HRT; Gambacciani, Ciaponi, Cappagli, Benussi, & Genazzani, 2000; Maki & Sundermann, 2009; Sherwin, 2005; Sørensen, Rosenfalck, Højgaard, & Ottesen, 2001). This suggests that muscle function, cognition and bone metabolism are primary candidates for studying the interaction between reproductive hormones and low energy availability.

The use of hormonal contraceptives (HCs), which deliver exogenous steroid hormones to the system and down-regulate endogenous reproductive hormone concentrations, may further confound the effects of low energy availability on these aspects of physiological function. Approximately 30% of the UK general population use HCs (Cea-Soriano, García Rodríguez, Machlitt, & Wallander, 2014), while previous estimates of oral contraceptive (OC) use in elite athletes range from 40 to 46% (Brynhildsen et al., 1997; Torstveit & Sundgot-Borgen, 2005). Elite athletes may be more inclined to use HCs than the general population in order to prevent fluctuations in performance associated with the menstrual cycle, or manipulate bleeding patterns around training and competition (Schaumberg et al., 2017). It is currently not clear why athletes initiate or discontinue HC use and the perceived side effects of HC use in this population are unknown. Furthermore, previous studies have detailed the proportion of OC use only, with no studies stratifying HC users by type (*i.e.,* progestin-only or combined), delivery method (*i.e.,* OC, implant, intra-uterine system [IUS], injection vaginal ring, transdermal patch) or preparation (brand). The steroid hormone content, concentration and delivery method may influence the physiological responses to HC use (Elliott-Sale et al., 2013; Godsland et al., 1990; Van Den Heuvel, Van Bragt, Alnabawy, & Kaptein, 2005). It is, therefore, important that HC use is well-characterised in elite athletes, so that future research can be designed according to the requirements of this population.

The two most common reproductive hormone models experienced by elite female athletes are the menstrual cycle and OC use, and these models can be used to investigate the effects of changing reproductive hormone concentrations (endogenous and exogenous) on muscle function, cognition and bone metabolism, with and without changes in energy availability. Previous research, without altered energy availability, has shown that muscle strength (Phillips, Sanderson, Birch, Bruce, & Woledge, 1996), cognitive performance in verbal fluency (Maki, Rich, & Shayna Rosenbaum, 2002) and verbal memory tasks (Maki et al., 2002), and concentrations of bone formation markers (Gass, Kagan, Kohles, & Martens, 2008) are highest during the ovulatory or mid luteal phases of the menstrual cycle, when oestrogen concentrations are elevated. In contrast, cognitive performance in tasks of mental rotation (Maki et al., 2002) and concentrations of bone resorption markers (Gass et al., 2008) have been shown to be highest when oestrogen is at its nadir during the early follicular phase of the menstrual cycle. Moreover, other studies have shown muscle strength, cognitive function and bone metabolism do not vary across the menstrual cycle (Elliott, Cable, Reilly, & Diver, 2003; Mordecai, Rubin, & Maki, 2008; Shimizu et al., 2009). This conflicting evidence may be a result of poor methodological design, as some of these studies have; 1) used inaccurate methods to determine menstrual cycle phase, without measuring reproductive hormone concentrations to confirm the endogenous milieu, 2) not controlled pre-trial diet and exercise, thereby introducing variability into measurement, or 3) used only two time points of the menstrual cycle, restricting the ability to differentiate the effects of oestrogen and progesterone. Further adequately controlled studies are required to assess the effects of the menstrual cycle on muscle function, cognition and bone metabolism, independent of energy availability.

Muscle strength and cognitive function have mostly been shown to remain unchanged between phases of an OC cycle (Elliott, Cable, & Reilly, 2005; Griksiene & Ruksenas, 2011), although some studies have shown improved verbal memory (Mordecai et al., 2008) or reduced muscle strength (Rechichi & Dawson, 2009) in the pill free interval (PFI). Participants in previous studies have used a variety of preparations, which have since been shown to increase inter-individual variability in oestradiol concentrations (Elliott-Sale et al., 2013) and increase the risk of type II errors. Several studies have measured bone metabolism across an OC cycle, although the metabolic markers recommended by the International Osteoporosis Foundation (Vasikaran et al., 2011), namely procollagen type 1 N propeptide (P1NP) and serum carboxy-terminal cross-linking telopeptide of type 1 collagen (β-CTX), are yet to be studied and consequently should be measured in order to clarify how these markers are affected across an OC cycle.

Few studies have assessed the effects of short term low energy availability on muscle and cognitive functions. Indeed, only two studies have assessed the effects of short-term ($\leq$ 2 weeks) energy restriction on muscle strength in females (Parkes, Belcastro, McCargar, & McKenzie, 1998; Zachwieja et al., 2001). These studies used moderate levels of energy restriction (75% required energy intake and 750 kcal·day^{-1} restriction), which is less restrictive than seen in many athletes, and participants took part in either ~100 min·day^{-1} mixed-modality exercise (Parkes et al., 1998) or 500 kcal·day^{-1} of treadmill running (Zachwieja et al., 2001). The effects of diet-induced energy restriction and exercise-induced energy restriction have not been studied separately. Further research is required to compare the muscle strength response to diet or exercise-induced low energy availability, whilst using a more severe level of energy restriction that is representative of the practices of athletes.

The effects of short-term energy restriction on cognitive performance have been studied on two occasions. Lieberman et al. (2008) showed that two days of a severely restrictive diet (183 kcal·day^{-1} energy intake), had no effect on cognitive performance compared to an energy balanced condition (2294 kcal·day^{-1} energy intake). A further study showed that grammatical reasoning and choice reaction time were improved after two days of calorie restriction (266 kcal·day^{-1} energy intake) compared to an energy balanced condition (3935 kcal·day^{-1} energy intake), with no effects on measures of vigilance and working memory (Lieberman et al., 2017). These studies included 2 h·day^{-1} (40-45% heart rate reserve; Lieberman et al., 2008) and 4 h·day^{-1} (40-65% $\dot{V}O_2$peak; Lieberman et al., 2017) low intensity exercise, which is representative of military training and not typical of athletes practices (Melin et al., 2015a). These protocols also resulted in large deviations from energy balance (-3681 kcal·day^{-1} and -2138 kcal·day^{-1}), which are equivalent to a negative energy availability, which have not been reported in athlete populations (Loucks et al., 2011; Melin et al., 2015). Further research is required that uses an energy availability more relevant to elite athletes and compares the effects of low energy availability achieved through either diet or exercise, as exercise is known to affect cognitive function (Chang, Labban, Gapin, & Etnier, 2012). Furthermore, only 7.4% (n = 2; Lieberman et al., 2008) and 26.1% (n = 6; Lieberman et al., 2017) of the population studied were female and Lieberman et al. (2017) showed that females were more susceptible to changes in cognitive function in response to energy restriction, illustrating the need for further research to be conducted in females. In order to determine whether reproductive hormones influence the response to low energy availability for muscle strength and cognition, as seen with bone health (Nattiv et al., 2007), studies should also compare the responses between the groups that represent different models of reproductive functioning, such as eumenorrheic women and OC users.

In order to address some of the discrepancies and gaps in previous research, the following aims of this thesis were generated:

1. Characterise the use of HCs in an elite female athlete population and identify the reasons for HC use, discontinuation and the perceived side effects of HC usage (Chapter 5).
2. Examine the effects of the menstrual cycle and OC use on muscle function, cognition and bone metabolism, when energy availability is not manipulated in recreationally active women (Chapters 6-8).
3. Examine the effects of low energy availability on muscle force and cognitive function in recreationally active women, to explore whether there are different responses dependent upon how the energy availability is achieved (*i.e.,* diet or exercise), and to assess if there are different responses between eumenorrheic women and OC users (Chapters 9-10).

Chapter 2.0. Review of literature

2.1. Introduction

The review of literature has been divided into five main sections. The first section (2.2) describes the models of reproductive function most pertinent to female athletes and their use in research. Section 2.3 reviews the consequences of low energy availability in female athletes, including the alterations to reproductive function, outlines the current models that describe this, and highlights areas that require further research. The subsequent sections discuss the roles of reproductive hormones and low energy availability on muscle function (section 2.4) and cognition (section 2.5), while section 2.6 explores the effects of reproductive hormones only on bone metabolism, as the effects of low energy availability on bone metabolism are summarised in section 2.3 and this does not form the basis of an experimental chapter in this thesis.

2.2. Models of reproductive function

Reproductive function changes across the lifespan in women. Reproductive hormone concentrations are low during childhood and early adolescence, until menarche occurs at ~11-16 years, when reproductive hormone concentrations are increased and fluctuate in a cyclical manner during the menstrual cycle (Norman, 2014). This typically occurs until menopause at ~45-55 years, whereby reproductive function ceases and reproductive hormone concentrations are reduced (Santoro & Randolph, 2011). Reproductive function can be altered by exogenous hormones such as HCs and HRT, environmental factors (*e.g.*, energy availability, stress etc.), disease (*e.g.*, polycystic ovary syndrome, thyroid dysfunction etc.), in-vitro fertilisation (IVF) treatment and pregnancy (Amato, Verghi, Nucera, Galluzzo, & Giordano, 2011; Dobson, Ghuman, Prabhakar, & Smith, 2003; Elliott et al., 2005b; Greising et al., 2009; Hilton & Loucks, 2000; Rivera et al., 1999). The most pertinent models of reproductive function to female athletes are the menstrual cycle (and disturbances to this) and HC use as the majority of athletes are post-menarche and pre-menopausal.

2.2.1. Menstrual cycle

The menstrual cycle is a complex process regulated by reproductive hormones, which are controlled by the hypothalamic-pituitary-ovarian axis. Gonadotropin releasing hormone (GnRH), produced by the hypothalamus, regulates the production of the gonadotropins follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary, which in turn mediate the production of oestrogens, progesterone and inhibin from the ovaries (Barbieri, 2014).

The first day of the menstrual cycle is indicated by the onset of menstruation (menstrual bleeding), which is the result of the shedding of the endometrial lining of the uterus. At the beginning of the menstrual cycle, GnRH pulse frequency results in the production of FSH, which stimulates the growth of primordial follicles in the ovary into a primary follicle (Reed & Carr, 2000). The primary follicles then develop into secondary and then tertiary follicles over the next ~14 days (the follicular phase), during which granulosa cells and thecal cells develop and proliferate (Ferin, Jewelewicz, & Warren, 1993). These cells produce the steroid hormone androstenedione, which is converted into oestrogen and results in a gradual rise in oestrogen concentrations over the follicular phase (Ferin et al., 1993). Increasing oestrogen concentrations inhibit FSH and LH production, preventing additional follicular development, and also stimulate further oestrogen synthesis in granulosa cells (Reed & Carr, 2000). Towards the end of the follicular phase, there is a rapid increase in oestrogen concentrations and granulosa cells also secrete inhibin and progesterone (Barbieri, 2014). High oestrogen concentrations and rising progesterone levels enhance pituitary responsiveness to GnRH, which results in a rapid increase in LH production known as the 'LH surge' (Reed & Carr, 2000). This LH surge is essential for ovulation and stimulates the release of the secondary oocyte (egg) from the tertiary follicle where it travels to the fallopian tubes for implantation (Ferin et al., 1993). The empty tertiary follicle collapses and is invaded by granulosa and thecal cells, a process called 'luteinisation', and produces an endocrine structure known as the corpus luteum. Lipids within the corpus luteum are used to synthesise steroid hormones and result in a large increase in progesterone concentrations and increased oestrogen concentrations (Ferin et al., 1993; Stricker et al., 2006). The primary function of increased progesterone concentrations is to prepare the uterus for pregnancy by stimulating the maturation of the uterine lining. The lifespan of the corpus luteum is ~12 days and if pregnancy does not occur, the corpus luteum undergoes apoptosis and becomes an inactive structure known as the corpus albicans and progesterone and oestrogen production is reduced (Reed & Carr, 2000). Declining progesterone concentrations cause the contraction of the blood vessels of the endometrium, resulting in the death of surface cells of the endometrium over the next 2 days, which are sloughed and result in a menstrual bleed (Barbieri, 2014). This is known as the luteal phase, which occurs between ovulation and the onset of menstruation and is typically 14 days in length (Reed & Carr, 2000). The declining oestrogen and progesterone concentrations at the end of the luteal phase halts negative feedback to the hypothalamus and anterior pituitary, resulting in increased FSH production, which begins the hormonal regulation of the following menstrual cycle (Ferin et al., 1993). The concentrations of reproductive hormones throughout a typical menstrual cycle are presented in Figure 2.1.

Side effects associated with the menstrual cycle, termed dysmenorrhea, include cramps, headaches, nausea and fatigue and are experienced by the majority (60-91%) of non-HC users in the general population (Ju, Jones, & Mishra, 2014), primarily in the days surrounding menstruation. Chantler, Mitchell and Fuller (2009) showed that dysmenorrhea symptoms impaired exercise performance and 51% of elite athletes (n = 90) perceived that their training and performance was affected by menstrual cycle-related symptoms (Bruinvels, Burden, Brown, Richards, & Pedlar, 2016), evidencing the importance of understanding these in athletic populations. Exercise may reduce dysmenorrhea symptoms (Brown & Brown, 2010; Daley, 2008), therefore the large training volumes undertaken by elite athletes may affect the prevalence or severity of symptoms in this population compared to non-exercisers. Despite this, the types of side effects experienced during the menstrual have not been studied in elite athletes and therefore research is required to show the prevalence of perceived side-effects in this population.
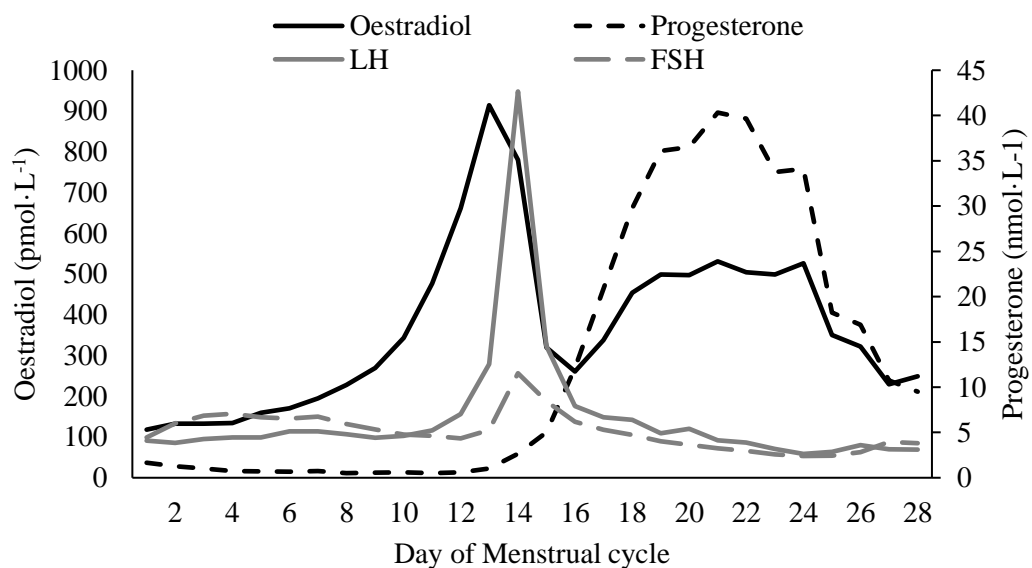
Figure 2.1. Oestradiol, progesterone, luteinising hormone (LH) and follicle stimulating hormone (FSH) concentrations during the follicular phase, ovulation and luteal phase of the menstrual cycle, adapted from Stricker et al. (2006).

2.2.2. Reproductive hormones

2.2.2.1. Oestrogen

Oestrogens are naturally occurring steroid hormones that exert their effects via oestrogen receptors (ER); ER-α, ER-β and G-coupled ER 1 (GPER-1; Eyster, 2016). ERs are present in the majority of human tissues (Jia, Dahlman-Wright, & Gustafsson, 2015) and oestrogen is considered integral to the physiological function of the brain, skeletal system, cardiovascular

system and reproductive system, among other areas (Almeida et al., 2017; Knowlton & Lee, 2012; Li & Shen, 2005; Reed & Carr, 2000). In addition to classic nuclear steroid hormone receptor actions mediated through ER-α and ER-β, oestrogens also act in a non-genomic manner and can exert rapid effects on cellular signalling pathways, such as altering intracellular calcium mobilisation and endothelial function (Dent, Fletcher, & McGuigan, 2012; Simoncini & Genazzani, 2003). Three naturally occurring oestrogens are produced endogenously during normal conditions; oestradiol, oestrone and oestriol. Of these, oestradiol (also known as 17-β-oestradiol) is the most biologically active and considered to be the most important in terms of physiological function (Bennink, 2004). Oestradiol is approximately three times more biologically active than oestrone and 16 times more biologically active than oestriol (Bennink, 2004), therefore oestradiol concentrations are typically the principal focus of investigations studying the effects of oestrogen.

The concentration of total oestradiol during the menstrual cycle is depicted in Figure 2.1, however this includes oestradiol bound to sex hormone-binding globulin (SHBG) and albumin, which is biologically inactive (Wu, Motohashi, Abdel-Rahman, Flickinger, & Mikhail, 1976). Only a small proportion (~1-3%) of oestradiol exists in its free, bioavailable form (Wu et al., 1976) and, while some studies show that this varies across the menstrual cycle in a similar manner to total oestradiol concentrations (Lin, Yoshida, & Sekiba, 1987; Linton et al., 2016; Thys-Jacobs, McMahon, & Bilezikian, 2008; Yeung et al., 2013), other studies have shown that concentrations do not significantly fluctuate throughout the menstrual cycle (Bao et al., 2003; Elliott, Cable, Reilly, & Diver, 2003).

2.2.2.2. Progesterone

Progesterone is a steroid hormone that principally exerts its effects on the nuclear progesterone receptors (PR) PR-A and PR-B (Kaya et al., 2015), which often produce opposing effects (Vegeto et al., 1993). Progesterone can also exert non-nuclear, rapid effects on cells through progesterone membrane receptors (Gellersen, Fernandes, & Brosens, 2008; Moussatche & Lyons, 2012; Singh, Su, & Ng, 2013). Whilst progesterone is not considered to influence physiological function to the extent of oestrogens, progesterone can exert numerous effects on the body, which are most apparent in the reproductive system, nervous system and brain (Moussatche & Lyons, 2012; Singh et al., 2013). Progesterone concentrations remain low (~1 nmol·L^{-1}) throughout the follicular phase of the menstrual cycle, and are significantly elevated in the luteal phase (Figure 2.1) to around 40 nmol·L^{-1}. The proportion of non-protein-bound progesterone is slightly lower in the follicular phase (3.4%) compared to the luteal phase (4%), however across the menstrual cycle, free progesterone concentrations correlate strongly with total progesterone (Minassian & Wu, 1993).

2.2.3. The menstrual cycle in research

The menstrual cycle is frequently used as a model to examine the effects of reproductive hormone concentrations on aspects of physiological function. In these studies, it is important that sampling time points are chosen that accurately represent the different phases and associated hormone concentrations across the menstrual cycle. The gold-standard approach would be to measure the relevant physiological outcome variable and reproductive hormone concentrations daily throughout the menstrual cycle, although this is expensive, intrusive and may not be appropriate if the outcome measure is affected by repeated assessment. A less frequent, periodic testing approach is commonly used, such as testing three times per week (Monday, Wednesday, Friday; Chiu et al., 1999; Gass, Kagan, Kohles, & Martens, 2008), or weekly (Phillips, Sanderson, Birch, Bruce, & Woledge, 1996) and these samples are then used to retrospectively classify participants into menstrual cycle phases. These methods however, may miss transient peaks in reproductive hormone concentrations (Stricker et al., 2006), or result in heterogeneity of reproductive hormone concentrations within phases (Phillips et al., 1996), especially in studies using longer periodic testing intervals. Therefore, the most common methodological approach is to prospectively identify days to conduct test sessions based upon physiological cues, such as menstruation or ovulation. There are, however, several methodological issues that need to be considered when prospectively identifying dates to conduct testing sessions, which are discussed below.

2.2.3.1. Identification of peak oestradiol concentrations

Many studies attempt to measure aspects of physiological function during the period immediately prior to ovulation, when oestrogen concentrations are highest (Stricker et al., 2006). In the absence of daily oestradiol assays that provide instantaneous feedback, experimenters are reliant on predicting the day of the menstrual cycle when peak concentrations will occur based upon estimations of cycle length or mapping of basal body temperature from previous cycles. Menstrual cycle length, and therefore the day of peak oestradiol concentrations relative to menstruation, varies between cycles (Fehring, Schneider, & Raviele, 2006), so this is not a reliable method to obtain peak oestradiol concentrations. Oestradiol concentrations measured either two days before or after peak concentrations occur can be up to two thirds lower than true maximal concentrations (Figure 2.1; Stricker et al., 2006). Several studies that have attempted to use this method have observed high inter-participant variability in oestradiol concentrations (Hertel, Williams, Olmsted-Kramer, Leidy, & Putukian, 2006; Janse De Jonge, Boot, Thom, Ruell, & Thompson, 2001). Instead, a stronger methodological approach is to use time points during the menstrual cycle that are based upon measureable, physiological set-points that reduce the variation between participants. For example, the early follicular phase (low oestrogen), post-ovulatory phase

(24h post LH surge; medium oestrogen) and mid-luteal phase (7-8 days post LH surge, high oestrogen).

2.2.3.2. Confirmation of ovulation

Over one third of menstrual cycles are anovulatory in a healthy female population and these are thought to occur intermittently in all women (Prior et al., 2015). Anovulatory cycles result in reduced concentrations of oestradiol and progesterone in the luteal phase (Hambridge et al., 2013) and therefore ovulation should be confirmed in order to represent a typical menstrual cycle reproductive hormone profile. Previous studies have shown that basal body temperature measurements correctly predict the date of ovulation in only 5% (McCarthy & Rockette, 1986) and 10% (Vermesh, Kletzky, Davajan, & Israel, 1987) of cases. Therefore, more suitable measures should be used, such as monitoring the LH surge using urinary testing kits, which is accurate for 91% of cycles (Behre et al., 2000).

2.2.3.3. Menstrual cycle length

The average menstrual cycle length is 28-29 days, although this varies substantially between and within individuals (Fehring et al., 2006; Stricker et al., 2006). In women (n = 276) that self-reported as having 'regular' menstrual cycles (between 21 and 35 days), there was a mean menstrual cycle length of 29.1 ± 3.5 days, although 46% of had a menstrual cycle length range > 7 days and 20% had a cycle range of > 14 days during a 30 week prospective measurement period (Creinin, Keverline, & Meyn, 2004). Menstrual cycles that are either short (< 21 days) or long (> 35 days) are typically considered irregular and may result in different reproductive hormone concentrations (Fehring et al., 2006). Experimenters should confirm that participants' menstrual cycle lengths are within normal ranges to reduce the effects of non-typical cycles on reproductive hormone concentrations.

2.2.3.4. Confirmation of hormone concentrations

Following identification of the testing dates within the menstrual cycle, experimenters should use hormonal assays to confirm that participants are in the expected phase of the menstrual cycle as variability in cycle length or incorrect identification of menstrual bleeding may result in reproductive hormone concentrations not being representative of the anticipated values. Gordon, Corbin & Lee (1986) demonstrated that 46% of women were not in the expected phase of the menstrual cycle based upon counting days from menstruation.

2.2.3.5. Time of day

Panico et al., (1990) showed that oestradiol concentrations were highest in the morning and decreased throughout the day, however other studies have shown that oestradiol has no

significant diurnal variation (Bungum, Franssohn, Bungum, Humaidan, & Giwercman, 2013; van Kerkhof et al., 2015). The diurnal rhythm has also been shown to vary across the menstrual cycle, where the highest concentrations of free oestradiol occurred later in the day in the early follicular phase (0822 ± 4.54 h) compared to the late follicular (0246 ± 3.06 h) and late-luteal phases (0346 ± 3.38 h; Bao et al., 2004). Therefore, given the potential for diurnal variation to affect hormone concentrations, it is important that assessments are conducted at the same time of day, both within and between participants.

2.2.3.6. Diet and exercise

Dietary constituents, such as alcohol (Schliep et al., 2012) and caffeine (Reichman et al., 1993), have been shown to influence oestradiol concentrations and exercise has been demonstrated to transiently increase circulating concentrations of oestradiol (Kraemer et al., 1995; Nakamura, Aizawa, Imai, Kono, & Mezaki, 2011). The effects of these factors on reproductive hormone concentrations can be limited by standardising diet and exercise pre-test.

2.2.3.7. Duration of eumenorrhea

It can take up to six months for normal reproductive function to resume following HC use (Nassaralla et al., 2011), therefore it is important that participants are free from HC use for six months prior to participation in research to prevent any effects of previous HC use on menstrual cycle function. Menstrual cycles typically resume approximately five months postpartum, although, as this is highly variable between participants, any participant less than one year postpartum should confirm ovulation, using a LH test kit, prior to participation (Holmberg-Marttila, Leino, & Sievänen, 2003; Li & Qiu, 2007).

2.2.3.8. Recommended protocol

Based upon the above considerations for conducting menstrual cycle-based research, the following menstrual cycle phases and experimental controls are recommended for research in this area:

1. Measure during the early follicular phase; as soon as possible following the onset of menstrual bleeding (low oestrogen, low progesterone);
2. Measure in the post-ovulatory phase, using LH urinary assays to confirm ovulation, and testing within 24h of the LH surge (medium oestrogen, low progesterone);
3. Measure in the mid-luteal phase, 7-8 days following the LH surge, based upon the consistent luteal phase length for ovulatory women (high oestrogen, high progesterone);
4. Confirm hormone concentrations using hormonal assays of oestradiol;

5. Control time of day, diet and exercise to reduce variations of reproductive hormone concentrations.

2.2.4. Hormonal contraceptives

Hormonal contraceptives are exogenous steroid hormones that are used to inhibit ovulation and prevent pregnancy (Rivera et al., 1999). Hormonal contraceptives are either combined combinations, consisting of a synthetic oestrogen and progestin, or progestin-only. The progestin component prevents ovulation by inhibiting the LH surge, whereas the oestrogen component provides negative feedback to the anterior pituitary to reduce FSH production and prevent the development of follicles (Diczfalusy, 1968; Swerdloff & Odell, 1969). There are several different delivery methods of HCs including the OC, IUS, implant, injection, transdermal patch and vaginal ring. Cea-Soriano et al., (2014) showed that 30% of UK women of reproductive age use HCs, with 54% of these women using combined OCs. In elite athletes, it has previously been reported that 40-46% of athletes use OCs (Brynhildsen et al., 1997; Torstveit & Sundgot-Borgen, 2005), which is greater than the general population, although the proportion that use combined or progestin-only preparations has not been stated and the prevalence of other, non-orally administered, preparations has also not been reported. Previous research (Cauci et al., 2016; Cauci et al. 2017) has reported the prevalence of different preparations of combined OC in Italian athletes, although only 14.6% and 18.7% of these populations were classified as elite (competing nationally or internationally), with overall prevalence of OC use (25.8% and 29.2%) similar to non-athletic populations.

In addition to providing contraception, HCs can offer non-contraceptive benefits such as reducing dysmenorrhea symptoms and altering the bleeding profile which may be beneficial for athletes when competing (Schindler, 2013). Schaumberg et al., (2017) showed that 73% of OC-using competitive athletes deliberately manipulated the timing of their menstruation in the previous year, with 54% of athletes manipulating their OC use due to sport competition. This highlights that the HC use of athletes may be different to the general population due to their unique requirement to train and compete, therefore further research is required to characterise HC use in this population, understand the reasons athletes choose to use HCs and identify the perceived positive and negative side effect of HC use.

2.2.4.1. Combined oral contraceptives

Combined OCs contain a synthetic oestrogen, principally ethinyl oestradiol (EO, also known as 17-α-oestradiol), and a progestin, of which there are many different types that vary in potency and androgenicity (Benagiano, Primiero, & Farris, 2004). The progestin used classifies OCs as first (*i.e.,* norethynodrel, norethindrone, ethynodiol diacetate), second (*i.e.,*

levonorgestrel, norethisterone), third (*i.e.,* norgestimate, desogestrel, gesotodene, cyproterone acetate) or fourth (drospirenone) generation OCs, which have been reported to produce different side effects (for review see Benagiano et al., 2004). Combined OC regimens typically consist of 21 pill taking days, followed by a 7-day PFI, repeated in a continuous manner, however several formulations use shorter PFIs or a longer pill consumption phase (Coffee et al., 2014; Spona et al., 1996). Oestradiol, FSH and LH are increased throughout the 7-day PFI (Van Der Spuy, Sohnius, Pienaar, & Schall, 1990; van Heusden & Fauser, 1999; Willis, Kuehl, Spiekerman, & Sulak, 2006) due to the withdrawal of negative feedback to the anterior pituitary. Endogenous oestradiol concentrations are ~3-4 fold higher on the 7th day of the PFI compared to the 1st pill-free day (van Heusden & Fauser, 1999) and decline in a linear manner once daily OC consumption is resumed, however concentrations can remain elevated for several days of pill consumption (Jung-Hoffmann, Heidt, & Kuhl, 1988; Spona et al., 1996). The circulating concentrations of EO and endogenous oestradiol throughout an OC cycle are displayed in Figure 2.2.
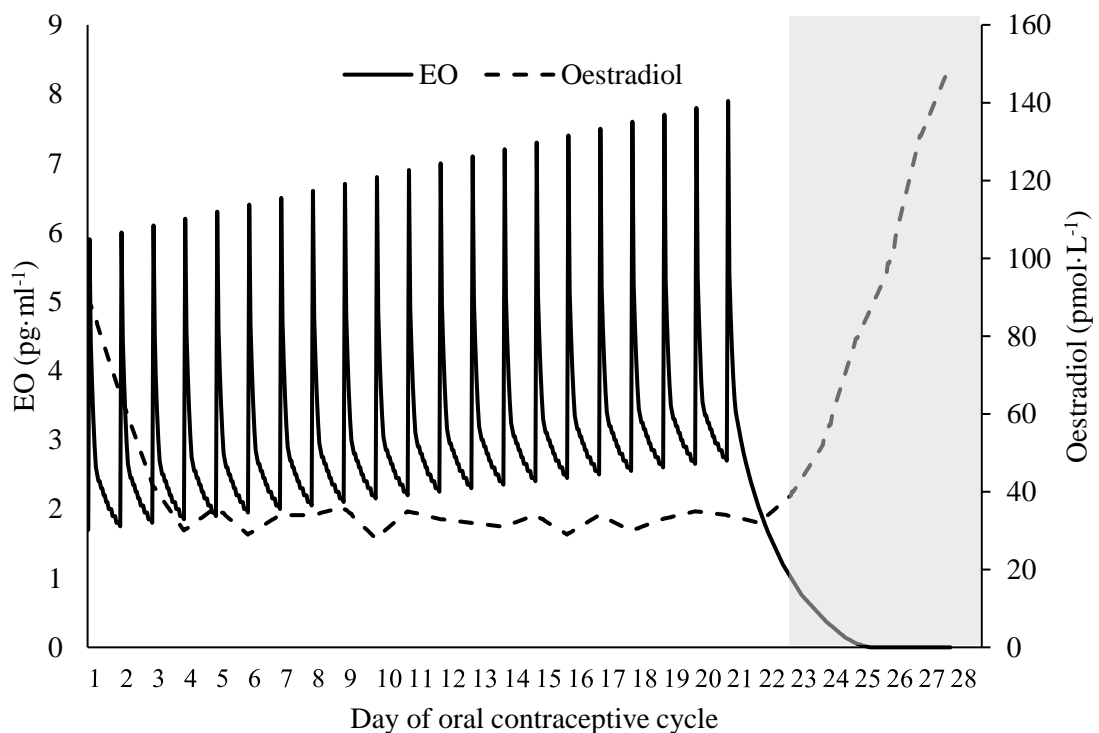
Figure 2.2. Ethinyl oestradiol (EO) and oestradiol concentrations during a combined oral contraceptive cycle. Grey area indicates the 7-day pill free interval. Adapted from Spona et al. (1996) and Carol et al. (1992).

16

The majority of women experience peak concentrations of EO ~60-90 minutes after OC ingestion (Kuhnz, Al-Yacoub, & Fuhrmeister, 1992; Figure 2.3), however there is a large degree in variability of responses; half-life for EO elimination ranged between 2.5 and 30 h, with a ten-fold variation between-participants in area under the curve for EO concentrations (Fotherby et al., 1981). The pharmacokinetics of EO are independent of the progestin co-administered (Dibbelt et al., 1991; Kuhnz, Back, Power, Schütt, & Louton, 1991; Orme et al., 1991). EO is mostly (~98%) bound to albumin and does not bind to SHBG, (Carol, Klinger, Jäger, Kasch, & Brandstädt, 1992; Kuhnz, Pfeffer, & al-Yacoub, 1990), which may explain its increased potency as SHBG-bound oestradiol is inactive. Levonorgestrel (LNG), the most widely use progestin, follows a similar pharmacokinetic profile to EO and is largely bound to SHBG (~65%) and albumin (~35%) (Kuhnz et al., 1992a). Concentrations of exogenous synthetic hormones accumulate over the course of an OC cycle, with peak EO (~52%) and LNG (123-153%), and area under the curve EO (75-87%) and LNG (261-273%) higher on the 21st day of pill consumption compared to the 1st day of consumption (Carol et al., 1992). Mean trough concentrations also increase throughout pill consumption days for LNG (Kuhnz, Al-Yacoub, & Fuhrmeister, 1992b) and EO (Dibbelt et al., 1991; Kuhnz et al., 1991) and reach a steady state after ~day 14 pill consumption, while SHBG levels increase by ~50% over the course of pill consumption days (Kuhnz et al., 1992a).
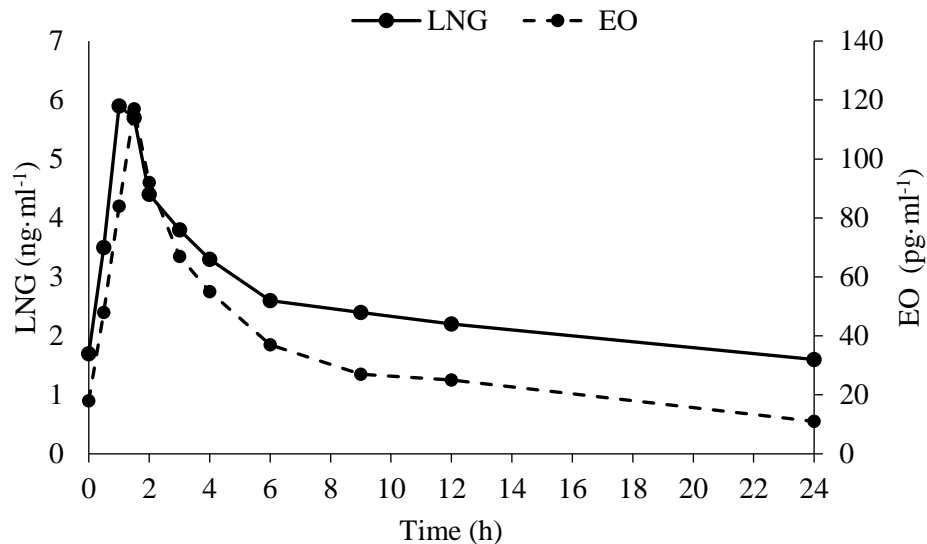
Figure 2.3. Pharmacokinetics of ethinyl oestradiol (EO) and levonorgestrel (LNG) concentrations in the 24 hours following pill consumption (150mg LNG, 30µg EO), adapted from Kuhnz et al. (1992a).

2.2.5. *The oral contraceptive cycle in research*

2.2.5.1. Phases of the oral contraceptive cycle

Due to the interaction between exogenous synthetic hormones and endogenous production of reproductive hormones, the OC cycle can be used to assess the effects of differing synthetic and naturally occurring reproductive hormone concentrations on aspects of physiological function. To examine this, many studies have compared pill consumption days to the PFI (Elliott, Cable, & Reilly, 2005; Mordecai, Rubin, & Maki, 2008; Zittermann et al., 2002) and some studies have assessed several time points during the PFI to encompass the increased endogenous oestradiol production during this period (Rechichi & Dawson, 2009). It is important to test participants in both the PFI and the pill consumption phase to identify the effects of EO and endogenous oestradiol on physiological function, however as EO concentrations increase during the pill consumption phase, test sessions conducted during the early and late pill consumption phases can be used to examine the effects of different exogenous hormone concentrations on physiological function.

2.2.5.2. Timing of pill consumption

The concentrations of synthetic oestrogens and progestins peak ~60-90 minutes after pill consumption, and then rapidly decline in a curvilinear manner over the next 24 hours (Kuhnz et al., 1992b). Standardisation of the timing of test sessions in relation to OC consumption can be used to minimise the variation of circulating synthetic hormone concentrations within and between participants.

2.2.5.3. Preparation of pill

Elliott-Sale et al., (2013) demonstrated that the concentration of endogenous oestradiol varies significantly between different preparations (brands) of OC that contain different synthetic oestrogens and progestins. Where possible, studies should recruit participants using the same preparation of OC to reduce inter-individual variation and minimise the possibility of type II errors occurring.

2.2.5.4. Duration of pill use

Side-effects indicative of improper cycle regulation, such as breakthrough bleeding and spotting, are present in ~25% of users in the first cycle of OC use, then gradually decline to <10% after 6 months, whereby the prevalence of these symptoms remains stable (Foidart, Wuttke, Bouw, Gerlinger, & Heithecker, 2000; Schrager, 2002). Recruiting participants that have used the same OC brand for a minimum of six months, as utilised in previous research (Elliott et al., 2005), will limit the effects associated with initial OC use on cycle regulation.

2.2.5.5. Recommended protocol

Based upon the above considerations for conducting research across an OC cycle, the following OC phases and experimental controls are recommended for research in this area:

1. Test during pill consumption phases and the PFI.
2. Test during the early and late pill consumption phases due to the different concentrations of circulating synthetic hormones.
3. The timing of OC consumption relative to test sessions should be standardised.
4. Where possible, participants should use the same preparation of OC.
5. Participants should have used their current OC for at least 6 months prior to participating.

2.3. Low energy availability in athletes

2.3.1. Energy availability

Energy availability is defined as the amount of energy available for the body to use, after that expended in exercise training, proportionate to lean body mass (LBM), with an energy availability of 45 kcal·kgLBM^{-1}·day^{-1} typically considered energy balanced (Loucks, Kiens, & Wright, 2011). Low energy availability may be caused by either 1) intentional behaviours to reduce weight, 2) compulsive weight control behaviour, such as disordered eating or pathological conditions, or 3) an inadvertent inability to match energy intake to energy demands (Nattiv et al., 2007). Table 2.1 shows the energy availability measures in free-living, female athletes.

2.3.2. Female athlete triad

The Triad was officially recognised by the American College of Sports Medicine in 1992 (Yeager, Agostini, Nattiv, & Drinkwater, 1993) and the first position stand on this issue was published several years later (Otis, Drinkwater, Johnson, Loucks, & Wilmore, 1997). Initially, the Triad was defined as a syndrome consisting of the interrelatedness of three conditions; disordered eating, amenorrhea and osteoporosis, which were commonly observed in female athletes and exercising females (Otis et al., 1997). An updated position stand in 2007 (Nattiv et al., 2007) highlighted that the three Triad conditions exist upon a spectrum (Figure 2.4), with each factor existing on a continuum between optimal health to sub-clinical and clinical conditions. Female athletes may present with one or more of these conditions and the time scale of these changes varies between factors; energy availability can be manipulated in days to weeks, menstrual function can be altered over several months, while bone mineral density (BMD) takes months or years to change (Nattiv et al., 2007). Disordered eating is no longer considered to be a prerequisite of the Triad, as it was understood that low energy availability

could be achieved inadvertently and sub-clinical menstrual disorders were included, as these have been linked to poor bone health (Nattiv et al., 2007). The most recent iteration of the Triad (De Souza et al., 2014) adheres to Triad conditions existing on a spectrum, but has also provided return-to-play recommendations and clinical guidelines for physicians and practitioners, which are based upon current evidence.
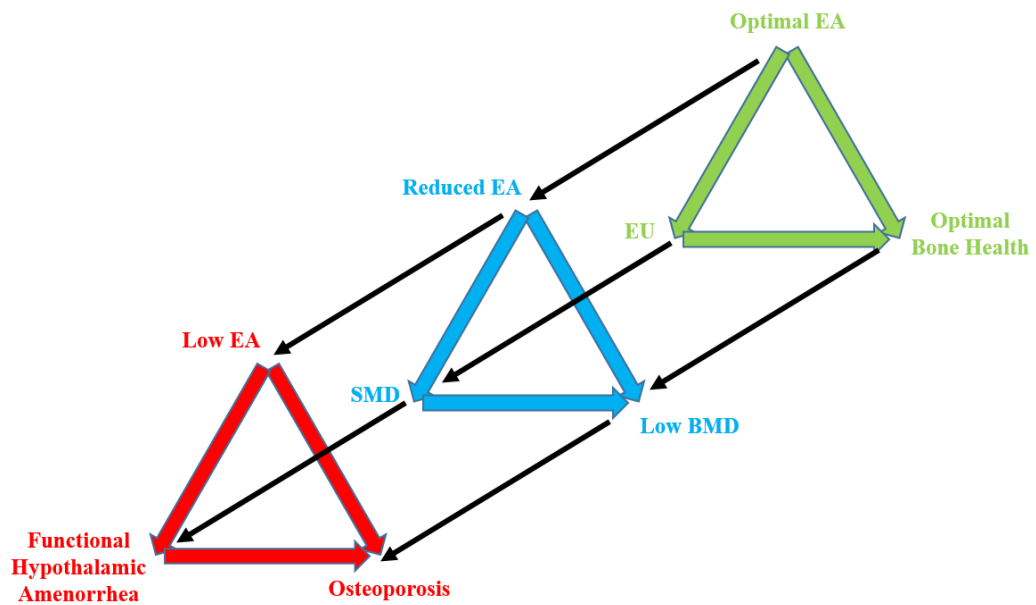
Figure 2.4. Components of the female athlete triad presented on a continuum from optimal health to the most serious clinical sequelae (adapted from Nattiv et al., 2007). Bone mineral density, BMD; energy availability, EA; eumenorrhea, EU; sub-clinical menstrual disorders, SMD.

Table 2.1. Study populations, time points and measurements of prospectively measured energy availability in free-living female athletic populations.

Author	Population	Monitoring period	LBM measurement	DEI measurement	EEE measurement	Energy availability
Brown et al. (2017)	25 pre-professional dancers	One week period during normal training	Skinfold measurements at 7 sites (Durnin & Womersley, 1974)	7-day prospective weighed food diary + 24 h recall	Accelerometer and METs	24 kcal·kgLBM^{-1}·day^{-1} (weekdays) 36 kcal·kgLBM^{-1}·day^{-1} (weekend)
Doyle and Lucas (2010a)	15 professional ballet dancers	4 day dietary period, estimated EEE	DXA scan	4-day weighed food diary	Estimated METS	3.75 kcal·kgLBM^{-1}·day^{-1} (41.1 kcal·kgLBM^{-1}·day^{-1} in controls)
Lagowska and Kapczuk (2016)	31 athletes (various sports) and 27 ballet dancers	3 day period during training	Bioelectrical impedance	7-day diet record with photo album assistance	Heart rate monitors and laboratory calculated $\dot{V}O_2$	28.3 kcal·kgLBM^{-1}·day^{-1} (athletes) 21.7 kcal·kgLBM^{-1}·day^{-1} (dancers)
Koehler et al., (2013)	185 young elite athletes	One week period during normal training	Bioelectrical impedance	7-day diet record of standardised foods	Training logs	29.6 kcal·kgLBM^{-1}·day^{-1}
Melin et al., (2016)	25 elite athletes in weight-sensitive sports	One week period during normal training	DXA scan	7-day prospective weighed food diary	Heart rate monitors and training logs	Mean = 42.5 kcal·kgLBM^{-1}·day^{-1} < 45 = 44% ≥ 45 = 56%
Melin et al. (2015)	40 elite endurance runners	One week period during normal training	DXA scan	7-day prospective weighed food diary	Heart rate monitors and training logs	Mean = 39.6 kcal·kgLBM^{-1}·day^{-1} ≥ 45 = 37.5% ≥ 30 and < 45 = 42.5% < 30 = 20%

Muia et al., (2016)	61 adolescent middle and long distance runners	5-day period (3 training days)	Skinfold measurement at 2 sites (Warner, Fornetti, Jallo, & Pivarnik, 2004)	5-day diet record (3 training, 2 non-training)	Training logs and RPE for calculation of METs	35.5 kcal·kgLBM^{-1}·day^{-1}
Reed et al., (2013)	19 NCAA Division I soccer players	3 consecutive training days pre, mid and post season	DXA scan	3-day prospective diet logs (non-weighed)	Heart rate monitors and training logs	43.5 kcal·kgLBM^{-1}·day^{-1} (pre) 35.2 kcal·kgLBM^{-1}·day^{-1} (mid) 44.5 kcal·kgLBM^{-1}·day^{-1} (post)
Schaal et al., (2011)		10 competitive, endurance trained athletes	DXA scan	7-day food record	Heart rate monitors and training RPE vs. laboratory $\dot{V}O_2$	29 kcal·kgLBM^{-1}·day^{-1} (CYC) 18 kcal·kgLBM^{-1}·day^{-1} (OVS)
Schaal et al. (2016)	9 national synchronised swimmers	4 days during normal training and after 2 and 4 weeks of intensified training	Skinfold measurements at 7 sites (Jackson & Pollock, 1985)	4 day prospective photo diary known food from cafeteria	Heart rate monitors	25.1 kcal·kgLBM^{-1}·day^{-1} (normal training) 17.8 kcal·kgLBM^{-1}·day^{-1} (intensified training)
Silva et al. (2015)	67 rhythmic gymnasts	1 and 4 days prior to international competition	Bioelectrical impedance	24 h record of dietary intake	Questionnaire of training volume (weekly)	31.5 kcal·kgLBM^{-1}·day^{-1}
VanHeest et al. (2014)	10 junior national swimmers	Every 2 weeks during 12 week training programme	Skinfold measurements at 7 sites (Durnin & Womersley, 1974)	3 day prospective weighed food diary + 24 h recall	Training logs and diaries	OVS (n=5) 11 kcal·kgLBM^{-1}·day^{-1} EU (n=5) 32 kcal·kgLBM^{-1}·day^{-1}
Viner et al. (2015)	4 professional cyclists	3 day period pre-season, mid-season and off-season	DXA scan	3 day prospective weighed food diary	Training logs and diaries	26.2 kcal·kgLBM^{-1}·day^{-1} (pre) 25.5 kcal·kgLBM^{-1}·day^{-1} (mid) 23.8 kcal·kgLBM^{-1}·day^{-1} (off)

Dietary Energy Intake, DEI; Duel-energy x-ray absorptiometry, DXA; Eumenorrheic, EU; Exercise Energy Expenditure, EEE; Lean Body Mass, LBM; Metabolic equivalents, MET; Ovarian supressed, OVS; Oxygen consumption ($\dot{V}O_2$).

2.3.2.1. Energy availability and menstrual function

Loucks and Heath (1994) provided seven female participants with a dietary energy intake of 45 kcal·kgLBM^{-1}·day^{-1} (balanced energy availability) or 10 kcal·kgLBM^{-1}·day^{-1} (low energy availability) for five days during the follicular phase of the menstrual cycle, whilst undertaking no exercise. On the final day of the protocol, LH pulse frequency was significantly reduced (23%) and LH pulse amplitude was significantly increased (40%) in the low energy availability condition. When the same levels of energy availability (10 and 45 kcal·kgLBM^{-1}·day^{-1}) were used, with the addition of 30 kcal·kgLBM^{-1}·day^{-1} of treadmill exercise in both conditions, similar reductions in LH pulse frequency and increases in LH pulse amplitude were shown in the low energy availability condition (Loucks, Verdun, & Heath, 1998). Importantly, despite a large volume of daily exercise, there were no changes in LH pulsatility in the balanced energy availability condition, suggesting that energy availability, not the stress of exercise, is the regulator of changes to the hypothalamic-pituitary-adrenal (HPA) axis. The frequency and amplitude of LH pulsatile secretions from the pituitary is a critical regulator of menstrual function (Reame et al., 1984) and therefore this provides causal evidence of the effects of energy availability on ovarian suppression.

Whilst undertaking exercise, equivalent to 15 kcal·kgLBM^{-1}·day^{-1}, for five days during the early follicular phase, LH pulsatility was not affected when energy availability was 45 or 30 kcal·kgLBM^{-1}·day^{-1}, yet pulsatile secretion patterns were significantly affected at an energy availability of 10 and 20 kcal·kgLBM^{-1}·day^{-1} (Loucks & Thuma, 2003). This suggests a threshold at which energy availability impairs HPA axis function, with ≥ 30 kcal·kgLBM^{-1}·day^{-1} not resulting in any significant changes in reproductive function. Recent evidence has opposed this, however, and showed that whilst there is no threshold of energy availability below which menstrual disturbances occur, menstrual disturbance frequency increases linearly as energy availability decreases (Lieberman, De Souza, Wagstaff, & Williams, 2018). Interestingly, the effects of low energy availability on LH pulsatility appear to be more pronounced in those with longer luteal phases (Loucks & Thuma, 2003) and a further study showed that LH pulsatility is not affected by low energy availability in women > 14 years of gynaecological age (Loucks, 2006). The importance of energy availability on reproductive status has been shown in several studies whereby increasing energy intake (Dueck, Matt, Manore, & Skinner, 1996; Kopp-Woodroffe, Manore, Dueck, Skinner, & Matt, 1999; Mallinson et al., 2013) or reducing training volume (Dueck et al., 1996; Kopp-Woodroffe et al., 1999) resulted in the resumption of menses in previously amenorrheic athletes.

Menstrual dysfunctions exist upon a spectrum, ranging from optimal, ovulatory cycles, to sub-clinical reproductive disturbances such as luteal phase defects (LPDs) and anovulatory cycles,

and to more serious clinical sequelae such as oligomenorrhea and amenorrhea (De Souza & Williams, 2004; see Table 2.2). Previous studies have shown that secondary amenorrhea is present in between 1 and 61% of exercising women, which is higher than in the general population (between 2 and 5%; Pettersson, Fries, & Nillius, 1973; Singh, 1981; Bachmann & Kemmann, 1982; De Souza et al., 2010). Sub-clinical menstrual disorders, such as anovulatory cycles and LPDs, occur in approximately half of female athletes (Broocks et al., 1990; De Souza et al., 2010; De Souza et al., 1998a) and menstrual dysfunction was present in 60% of 40 elite endurance athletes (oligomenorrhea, n = 6; primary amenorrhea, n = 4; secondary amenorrhea, n = 14), of which 67% had low (< 30 kcal·kgLBM^{-1}·day^{-1}) or reduced ($\geq$ 30 and < 45 kcal·kgLBM^{-1}·day^{-1}) energy availability (Melin et al., 2015). The magnitude of daily energy deficits has been linearly related to the frequency of menstrual disturbances (Williams et al., 2015) and it has previously been shown that calorie restriction within one cycle can result in LPDs (Pirke et al., 1985; Schweiger et al., 1987; Pirke et al., 1989), evidencing the speed at which nutritional status can impact hormonal function.

Table 2.2. Definitions and criteria for categorisation of menstrual function/dysfunction

Reproductive function	Definition
Eumenorrhea	Regular, ovulatory cycle, with luteal phase >10 days and cycle length 22-35 days
Luteal phase defects	Luteal phase $\leq$ 10 days, with reduced progesterone concentrations
Anovulatory cycles	Cycles where ovulation does not occur, defined by low LH and FSH secretion and reduced oestradiol
Oligomenorrhea	Irregular and inconsistent menstrual cycles ranging from 36-90 days in length
Primary amenorrhea	Menarche occurring after age of 16 years
Secondary amenorrhea	No menses for > 3 months

Luteinising hormone, LH; Follicle stimulating hormone, FSH

The mechanism by which low energy availability affects menstrual function is not fully understood. Alterations to the secretion patterns of gonadotrophins have been associated with increased ghrelin, and reduced leptin concentrations (Ackerman et al., 2012), and amenorrheic athletes have higher fasting ghrelin and lower leptin concentrations than eumenorrheic athletes and controls (Christo et al., 2008). Leptin has previously been shown to act indirectly on GnRH-secreting cells via actions on leptin-responsive afferent neurons (Donato et al., 2011; Finn et al., 1998; Quennell et al., 2009) and leptin administration has been shown to reverse

functional hypothalamic amenorrhea (Chou et al., 2011). Leptin has been identified as a signal of energy availability to the neuroendocrine axis when in an energy deficient state, which causes changes in LH pulsatility (Chan & Mantzoros, 2005), providing a mechanism by which leptin affects menstrual function. Ghrelin, which is increased during periods of low energy availability, is suggested to be a mechanism by which energy availability influences menstrual function as ghrelin administration has been shown to alter LH pulsatility (Kluge, Schüssler, Schmidt, Uhr, & Steiger, 2012). Ghrelin was negatively associated with LH secretion patterns in amenorrheic and eumenorrheic athletes (Ackerman et al., 2012), and non-athletes during periods of energy deficit (Scheid, De Souza, Hill, Leidy, & Williams, 2013). It is currently not clear if leptin, ghrelin, other adipokines such as Peptide YY, or a combination of these are responsible for changes in LH pulsatility, although these are the most likely neuroendocrine factors for communicating nutritional status to the hypothalamus (Scheid & De Souza, 2010). It is clear that reproductive function is impaired by short and long term reductions in energy availability, with LH pulsatility, the critical regulator of menstrual function, impaired with low energy availability. These clinical and sub-clinical changes in reproductive hormone concentrations, secondary to low energy availability, may have consequences for the health and performance of female athletes (De Souza et al., 2014).

2.3.2.2. Energy availability and a hypometabolic state

Energy conservation and reduced REE have been shown in amenorrheic athletes (De Souza & Williams, 2004; Laughlin & Yen, 1996) and amenorrheic runners have reduced resting metabolic rate compared to eumenorrheic runners (Myerson et al., 1991). In case studies, REE was increased in previously amenorrheic women following increases in calorie intake and the resumption of menses (Mallinson et al., 2013). Over the course of a 12-week training period, it was shown that junior swimmers who maintained menstrual cycle function (energy availability ~32 kcal·kgLBM^{-1}·day^{-1}), had similar to predicted REE values, whereas ovarian supressed swimmers' (energy availability ~11 kcal·kgLBM^{-1}·day^{-1}) REE was 19.7% lower than predicted values (Vanheest, Rodgers, Mahoney, & De Souza, 2014). The ratio of predicted REE to actual REE has been used to classify individuals as energy deficient or energy replete, with < 90% predicted REE used as an indicator of a hypometabolic state (De Souza et al., 2008). Exercising women classified as having a high drive-for-thinness (n = 27), based upon scores (≥ 7; 25th percentile) from the Eating Disorder Inventory-2, had a lower predicted REE:REE ratio (0.85) than women with normal drive-for-thinness (Gibbs, Williams, Scheid, Toombs, & De Souza, 2011). Reductions in REE are typically associated with reduced total T_3 concentrations (De Souza et al., 2008; Laughlin & Yen, 1996; Vanheest et al., 2014), a critical regulator of energy metabolism, which provides a potential mechanism by which this adaptive response occurs (McAninch & Bianco, 2014).

Adaptive responses in endocrine function have been identified in many studies of athletes with functional hypothalamic amenorrhea or in women during periods of low energy availability. These include reductions in total T_3 (Ihle & Loucks, 2004; Laughlin & Yen, 1996; Loucks et al., 1992; Loucks & Callister, 1993), leptin (Grinspoon, Hayden, Landt, & Nathan, 1997; Hilton & Loucks, 2000; Laughlin & Yen, 1997; Matejek et al., 1999), insulin (Grinspoon et al., 1997; Laughlin & Yen, 1996; Loucks et al., 1998), insulin-like growth factor 1 (IGF-1; Laughlin & Yen, 1996; Ihle & Loucks, 2004), glucose (Laughlin & Yen, 1996; Loucks et al., 1998) and increased growth hormone (GH; Laughlin & Yen, 1996; Loucks et al., 1998), ghrelin (Ackerman et al., 2012) and cortisol (De Souza et al., 1991; Laughlin & Yen, 1996; Loucks et al., 1998) concentrations. De Souza et al., (2003) also showed that adaptations representative of a hypometabolic state, such as reduced total T_3 and leptin concentrations, are present in active women that have subtle, sub-clinical menstrual disorders, such as LPDs, despite regular menstrual cycles.

In a dose-response study of energy balance (45 kcal·kgLBM^{-1}·day^{-1}) and three levels of low energy availability (10, 20 and 30 kcal·kgLBM^{-1}·day^{-1}), Ihle and Loucks (2004) demonstrated that insulin declined in a linear manner with declining energy availability, while oestradiol, T_3 and IGF-1 were relatively unaffected at moderate levels of energy availability (30 kcal·kgLBM^{-1}·day^{-1}), but were significantly reduced at more severe levels of low energy availability (10 and 20 kcal·kgLBM^{-1}·day^{-1}). This evidence suggests that the severity of reductions in energy availability affects the hormonal and metabolic environment, which may influence the physiological responses to periods of low energy availability.

2.3.2.3. Energy availability and bone

The combination of reduced reproductive hormone concentrations and other endocrine and metabolic disturbances due to reductions in energy availability may result in negative perturbations to bone (De Souza et al., 2014; Nattiv et al., 2007). Bone mineral density is typically assessed using duel-energy x-ray absorptiometry (DXA) scanning, with BMD T scores of -1.0 to -2.5 indicative of osteopenia and < -2.5 indicative of osteoporosis (Siris al, 2014). The current bone metabolic environment can also be assessed using bone metabolic markers, which can provide an indication of rates of bone formation and resorption, which may impact BMD in the long-term (Vasikaran, 2008).

2.3.2.4. Bone mineral density

The importance of menstrual function for athletes' bone health was first identified by Drinkwater et al., (1984), where lumbar vertebrae BMD was significantly lower in amenorrheic athletes compared to eumenorrheic athletes. Over a 15.5 month period, amenorrheic athletes who resumed menses had improved lumbar BMD (+6.3%), whilst those that remained amenorrheic continued to lose BMD (-3.4%) (Drinkwater, Nilson, Ott, & Chesnut, 1986). Whilst restoration of menstrual function can improve BMD, follow-up studies of two (Jonnavithula, Warren, Fox, & Lazaro, 1993) and eight (Keen & Drinkwater, 1997) years have shown that BMD is still lower in previously amenorrheic athletes compared to eumenorrheic athletes and controls. Bone mineral density is greater (1.27 $g \cdot cm^2$) in athletes with a history of optimal menstrual function compared to those with a history of oligomenorrhea/amenorrhea (1.18 $g \cdot cm^2$), and BMD was lowest (1.05 $g \cdot cm^2$) in athletes who had never had regular menstrual function (Drinkwater, Bruemner, & Chesnut, 1990). Without treatment, BMD is estimated to decline by 2-3% per year in amenorrheic women (De Souza et al., 2014; Misra et al., 2008) and BMD is negatively associated with the duration of menstrual dysfunction (Lloyd et al., 1988; Drinkwater et al., 1990; Myburgh et al., 1993). Independent of training volume, stress fractures are more prevalent in women with menstrual dysfunction compared to those with regular menstrual cycles (Bennell, Matheson, Meeuwisse, & Brukner, 1999; Duckham et al., 2012; Warren, & Hamilton, 1986) and whole-body fracture risk is increased by 50% for each SD decrease in age-adjusted BMD of the hip, spine or forearm (Marshall, Johnell, & Wedel, 1996; McClung, 2005).

Grinspoon et al. (1999) showed that lumbar spine, hip and total BMD were significantly lower in normal-weight women with hypothalamic amenorrhea compared to controls. Furthermore, anorexia nervosa patients had lower BMD than women with hypothalamic amenorrhea and controls, despite amenorrheic participants being matched for duration of amenorrhea and age of menarche, evidencing the additive effects of reproductive hormone suppression and low energy availability. Reduced BMD in amenorrheic athletes has been associated with lower resting metabolic rates (Doyle-Lucas et al., 2010a; Kaufman et al., 2002; Melin et al., 2015a) and reduced concentrations of leptin (Kaufman et al., 2002), T_3 (Melin et al., 2015a) and IGF-1 (Christo et al., 2008), suggesting a role of these factors in reduced bone health, although a causal role has not yet been identified for these factors.

2.3.2.5. Bone metabolism

Ihle and Loucks (2004) studied the response of bone metabolic markers during five days of a balanced (45 kcal·kgLBM^{-1}·day^{-1}) energy availability compared to three distinct levels of low energy availability (10, 20 and 30 kcal·kgLBM^{-1}·day^{-1}) in pre-menopausal women. Bone

formation markers, carboxy-terminal propeptide of type 1 procollagen (P1CP) and total osteocalcin, were supressed at all levels of low energy availability. P1CP declined linearly with energy availability, whilst osteocalcin was predominantly reduced between 20 and 30 $kcal \cdot kgLBM^{-1} \cdot day^{-1}$, suggesting potential differences in the mechanisms by which collagen formation and matrix mineralisation are affected. Changes in P1CP were linearly related to insulin and changes in osteocalcin were associated with T_3 and IGF-1 concentrations (Ihle & Loucks, 2004). Bone resorption, as assessed by N-terminal telopeptide (NTX) concentrations, was increased only at the most severe level of low energy availability ($10 \ kcal \cdot kgLBM^{-1} \cdot day^{-1}$), and was inversely related to oestradiol, suggesting possible oestrogen-dependent effects of energy availability on bone resorption. These data are supported by increased bone resorption marker concentrations in energy deficient women (De Souza et al., 2008) and an inverse relationship between BMD and NTX in amenorrheic women (Grinspoon et al., 1999), although other studies have shown reduced concentrations of urinary resorption markers (pyridinoline [PYD], deoxypyridinoline [DPD]) in amenorrheic runners compared to eumenorrheic runners and sedentary controls (Zanker & Swaine, 1998a, 1998b). Bone formation makers (osteocalcin, P1CP, bone-specific alkaline phosphatase [BAP]) were also reduced in amenorrheic runners, which was associated with reduced oestradiol, T_3 and IGF-1 concentrations (Zanker & Swaine, 1998a, 1998b). Energy availability has been shown to impact bone metabolism, although the importance of reproductive hormone status during periods of low energy availability is less well-known.

De Souza et al. (2008) categorised 44 exercising women by energy status (deficient or replete) and oestrogen status (deficient or replete). Women that were energy and oestrogen deficient (n = 8) exhibited the lowest P1NP and T_3 concentrations and the highest β-CTX and ghrelin concentrations, indicating the most severe metabolic perturbations. The lowest levels of bone formation in this group may be representative of the synergistic effects of deficiencies in oestrogen and energy, although it may also be due to these athletes displaying the greatest level of energy deficiency (lowest predicted REE:REE), which was significantly lower than the energy deficient, yet oestrogen replete women (n = 7). Interestingly, regardless of oestrogen status, perturbations in bone metabolism were not shown in energy-replete women, while bone metabolism was negatively affected in oestrogen-replete women with an energy deficiency, suggesting that energy availability may be more important than oestrogen status. The most severe outcomes are apparent in amenorrheic and energy deficient states, highlighting the inter-relatedness of energy availability, menstrual function and bone metabolism.

2.3.2.6. Prevalence of triad conditions

In a review of 65 studies that assessed either the prevalence of individual or combined components of the Triad, it was shown that the prevalence of the three Triad conditions existing simultaneously was relatively low, being 0-16% (Gibbs et al., 2013). The prevalence was higher for the simultaneous presentation of two Triad conditions (3-27%) and the presence of one Triad condition (16-60%). There are, however, methodological difficulties with these studies. The majority of studies have used self-reported menstrual function, which is unable to determine sub-clinical menstrual disorders such as LPDs or anovulation (De Souza et al., 1998b) and therefore may underestimate the occurrence of sub-clinical menstrual disorders. Furthermore, energy availability was typically assessed using ancillary measures, such as the presence of disordered eating, and was only directly assessed in one study (Hoch et al., 2009). In studies that measured BMD, 0-15% of athletes has a z score < -2.0 and 0-40% of athletes had a score < 1.0 (Gibbs et al., 2013). This however, may underrepresent the prevalence of poor bone health, as negative aberrations to BMD may take several years to manifest and the exercising population are expected to have greater BMD due to impact exercise.

Following this review (Gibbs et al., 2013), several studies have directly assessed energy availability in the female athlete population and the results are presented in Table 2.2. Mean energy availability was 11-17.8 kcal·kgLBM^{-1}·day^{-1} in ovarian supressed swimmers (Vanheest et al., 2014) and synchronised swimmers (Schaal, Tiollier, Le Meur, Casazza, & Hausswirth, 2016), and was also low (< 30 kcal·kgLBM^{-1}·day^{-1}) in dancers during training (Brown, Howatson, Quin, Redding, & Stevenson, 2017). Studies have primarily measured LBM using skinfold assessments (Brown et al., 2017; Muia et al., 2016; Schaal et al., 2016; Vanheest et al., 2014) and bioelectric impedance analysis (Łagowska & Kapczuk, 2016; Silva & Paiva, 2015), with few studies using gold-standard DXA scans (Melin et al., 2015b, 2016; Viner, Harris, Berning, & Meyer, 2015), as recommended by the Triad coalition (De Souza et al., 2014). Melin et al. (2015b) demonstrated that the three Triad conditions existed concurrently in 23% of elite endurance athletes (n = 40) and low (≥ 30 and < 45 kcal.kg.LBM^{-1}·day^{-1}) or reduced (<30 kcal·kgLBM^{-1}·day^{-1}) energy availability was present in 63% of athletes. Sixty percent of athletes had either clinical or sub-clinical menstrual dysfunction and reduced BMD was observed in 45% of athletes. While this study is limited by a lack of assessment of reproductive hormones across a complete cycle to evaluate menstrual function, this still provides evidence that the Triad conditions are prevalent in weight-sensitive sports such as endurance sports.

2.3.3. Relative energy deficiency in sport

In 2014, the International Olympic Committee released a consensus statement on the RED-S model, aiming to extend the current knowledge and understanding of the Triad (Mountjoy et al., 2014). The RED-S model suggests that, rather than a Triad of three components, relative energy deficiency is the initiating factor that may affect many other areas of health and performance, not limited to menstrual function and bone health (Mountjoy et al., 2014). Furthermore, male, non-Caucasian and disabled athletes may be affected in addition to female athletes (Mountjoy et al., 2014). These other potential factors are presented in Figure 2.5 and include immune function, cardiovascular health, metabolic rate, decreased muscle strength and aspects related to impaired cognition.

Low energy availability has been demonstrated to affect several of these factors including reduced muscle protein synthesis (Areta et al., 2013), endothelial dysfunction (Rickenlund, Eriksson, Schenck-Gustafsson, & Hirschberg, 2005), negative alterations to metabolism (Loucks & Thuma, 2003), impaired immune function (Hagmar, Hirschberg, Berglund, & Berglund, 2008), increased injury risk (Thein-Nissenbaum, Rauh, Carr, Loud, & McGuine, 2011) and a reduced training response and impaired performance (Vanheest et al., 2014). Despite this, many of the proposed factors in RED-S do not have a large evidence base or are partially based upon anecdotal clinical observations (De Souza et al., 2014; Mountjoy et al., 2014).

Since the initial RED-S paper (Mountjoy et al., 2014), further research has been published to evidence some of the putative links between low energy availability and the factors outlined in the RED-S model. In a large cross-sectional study, Ackerman et al. (2018) studied the associations between energy availability and RED-S symptoms in 1000 active females aged 15-30 years. Athletes identified as having an eating disorder/disordered eating in one or more of three administered questionnaires, were classified as having low energy availability, while those with no positive indication of eating disorders/disordered eating were classified as having adequate energy availability. Those classified as low energy availability (n = 473) were more likely to have negative side effects associated with RED-S in a wide range of self-reported measures. For the health-related consequences of RED-S (Figure 2.5A), only endocrine, growth and development and immunological function were not different between the low and adequate energy availability groups; all other negative health consequences occurred more frequently in the low energy availability group (odds ratio 1.50 – 3.01). Injury risk was the only RED-S performance parameter (Figure 2.5B) not different between energy availability groups, with impaired judgement, decreased coordination and decreased concentration occurring 4.33, 1.58 and 2.01 times more often in the low energy availability

group. Muscle strength and glycogen storage were not assessed as these are the most difficult to accurately determine using self-report methods.

This research indicates, in a large sample of female athletes, that low energy availability is associated with a number of the proposed consequences of RED-S. It must be noted, however, that the categorisation of low or adequate energy availability was based upon a positive indication of an eating disorder/disordered eating in one or more of three administered questionnaires, rather than direct quantification of energy availability. These three questionnaires showed a wide range of positive low energy availability identifications (20.1, 39.1 and 12.3 %) and while these are associated with risk of low energy availability, this may have overestimated the proportion with actual low energy availability in this population as acknowledged by the authors (Ackerman et al., 2018). Furthermore, confirmation of RED-S consequences either relied upon an existing diagnosis, or were self-reported, which may not accurately represent the prevalence of these conditions. Further research is required in a controlled setting, in order to provide evidence for a causal effect of low energy availability on perturbations to the health and performance of athletes in these suggested factors (De Souza et al., 2014; Mountjoy et al., 2015; Ackerman et al., 2018).

The Triad recognises that a combination of low energy availability and menstrual function, act in concert to impair bone health, with the poorest outcomes observed in females who are both energy deficient and oestrogen deficient (De Souza et al., 2008; Nattiv et al., 2007). In contrast, the current RED-S model proposes that an energy deficiency is the initiating, and most important, factor which may adversely affect aspects of physiological function (Mountjoy et al., 2014). This may understate the important role that changes in reproductive function such as amenorrhea, secondary to a low energy availability, could have on a number of aspects of physiological function (De Souza et al., 2014). For example, recent research has shown that amenorrheic athletes have poorer muscle strength, endurance and reaction time (Tornberg et al., 2017) and verbal memory and executive function (Baskaran et al., 2017) compared to eumenorrheic athletes. While this research suggests a potential effect of low energy availability on cognitive and muscle performance, these cross-sectional studies are unable to differentiate the potential effects of energy availability from menstrual status, so further prospective studies are needed to identify the independent effects of these factors. This will allow the determination of whether menstrual dysfunction acts in a synergistic or antagonistic manner to low energy availability and exacerbates perturbations to physiological function. It is particularly important to target research towards areas where existing evidence shows that alterations to reproductive function can impair physiological function. For example, the menopause represents an altered reproductive hormonal profile similar to hypothalamic

amenorrhea, and has been shown to accelerate the rate of sarcopenia (Messier et al., 2011), the cognitive decline (Farrag et al., 2002) and osteoporosis (Finkelstein et al., 2008), which can be ameliorated with HRT (Marco Gambacciani & Levancini, 2014; Maki & Sundermann, 2009; Sherwin, 2005; Sørensen et al., 2001). This suggests that reproductive hormones are vital for these aspects of physiological function and subsequently, these three areas of physiological function are the focus of this thesis. Therefore, the following sections of the literature will separately review the effects of reproductive hormones and low energy availability on muscle function, cognition and bone metabolism.
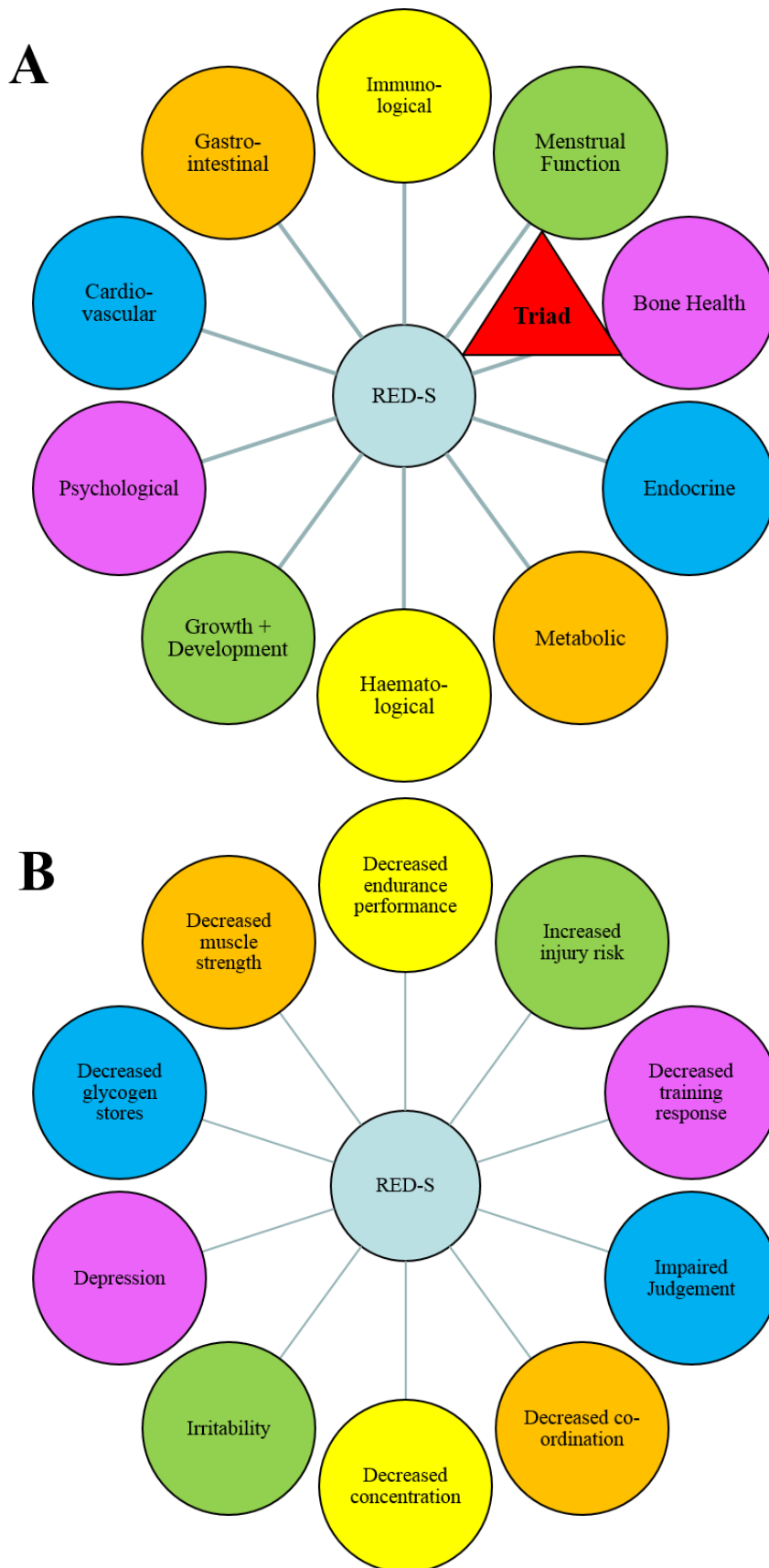
Figure 2.5. Health (A) and Performance (B) factors purportedly affected by a relative energy deficiency in sport (RED-S), adapted from Mountjoy et al. (2014).

2.4. Muscle function

Reduced muscle strength has been identified as a potential consequence of a low energy availability (Mountjoy et al., 2014), yet there is currently little evidence available to support this (De Souza et al., 2014). Furthermore, it is currently unclear whether alterations to the reproductive axis, alongside a low energy availability, impacts muscle strength. The following section will review literature studying the role of reproductive hormones and energy availability on muscle strength.

2.4.1. Reproductive hormones and muscle strength

The effect of reproductive hormones on muscle strength has been studied during the menstrual cycle (Cable & Elliott, 2004), pregnancy (Elliott et al., 2005), IVF treatment (Elliott et al., 2005b; Greeves, Cable, Luckas, Reilly, & Biljan, 1997), menopause (Phillips, Rook, Siddle, Bruce, & Woledge, 1993), HRT (Greeves, Cable, Reilly, & Kingsland, 1999) and OC use (Rechichi & Dawson, 2009). Longitudinal changes in body composition and muscle morphology with ageing and pregnancy confound the interpretation of the effects of reproductive hormones in pregnancy, menopause and HRT studies. Therefore, this literature review will focus on studies assessing IVF treatment, the menstrual cycle (Table 2.3) and OC use (Table 2.4) on the acute effects of reproductive hormones on muscle strength.

2.4.1.1. Potential mechanisms of reproductive hormones affecting muscle strength

Oestrogen rapidly increases intracellular calcium concentrations via its actions on membrane oestrogen receptors in a number of tissues (Morley, Whitfield, Vanderhyden, Tsang, & Schwartz, 1992; Younglai, Wu, Kwan, & Kwan, 2005). Membrane oestrogen receptors are present in skeletal muscle (Liu et al., 2009), which may provide a potential mechanism by which oestrogen influences muscle force, as increased calcium mobilisation would enhance actin and myosin binding (Dent et al., 2012). The non-genomic actions of oestrogen on skeletal muscle calcium concentrations have not yet been studied, so the mechanism by which oestrogen affects muscle force production, if any, is still unknown.

Reproductive hormones may also affect muscle strength through actions on cortical tract excitability, which is regulated through γ-aminobutyric acid (GABA) transmissions and is vital to the production of maximal force (Gandevia, 2001). Oestradiol binds to ERα sites on GABA releasing neurons (Schultz et al., 2009) and cortical tract excitability has been shown to be increased during the late follicular phase compared to the early follicular and mid-luteal phases of the menstrual cycle (Smith et al., 1999; Smith, Adams, Schmidt, Rubinow, & Wassermann, 2002). Corticospinal tract excitability is lowest during the mid-luteal phase

(Smith et al., 2002), which also suggests a suppressive role of progesterone, as progesterone metabolites have previously been shown to directly activate GABA$_A$ receptors (Callachan et al., 1987).

2.4.2. In-vitro fertilisation

IVF has only been used to assess the role of reproductive hormones on muscle strength in two previous studies (Elliott et al., 2005; Greeves et al., 1997). Greeves et al. (1997) studied first dorsal interosseus (FDI) muscle force production at two phases of IVF treatment; during a hypoestrogenic state when oestrogen was down-regulated (10-100 pmol·L^{-1}) using GnRH agonists and 9 days later when oestrogen was at supra-physiological levels (1551-9935 pmol·L^{-1}) following gonadotrophin injections. Despite significantly different reproductive hormone concentrations, there were no differences in FDI muscle maximal voluntary isometric force (MVIF) or fatigability. In a similar study, Elliott et al. (2005a) measured these time points in addition to further phases following human chorionic gonadotrophin administration and natural progesterone supplementation, which resulted in high (4041 pmol·L^{-1}) and relatively high (1535 pmol·L^{-1}) oestradiol concentrations. This study also showed no significant differences between these phases for FDI muscle MVIF. This evidence shows that even at supra-physiological oestrogen concentrations, there were no effects of oestrogen on muscle force production of the FDI muscle.

2.4.3. Menstrual cycle

2.4.3.1 Studies that have shown that muscle force changes across the menstrual cycle

Wirth and Lohman (1982) showed that grip strength was higher in the mid-follicular phase (days 6-10) compared to the luteal phase (days 19-24), while Davies et al. (1991b), showed grip strength and vertical jump height were higher in the early follicular phase (days 1-4) compared to the late follicular (days 10-12) and mid-luteal (days 19-21) phases. In these studies, menstrual cycle phases were calculated based upon counting days from menstruation and were not confirmed by hormonal analyses and therefore may not accurately reflect the desired reproductive hormone concentrations (Wideman, Montgomery, Levine, Beynnon, & Shultz, 2013).

Subsequent studies have examined muscle force production by taking periodic measurements and retrospectively classifying test dates into phases. Sarwar et al. (1996) measured MVIF of the quadriceps and handgrip strength weekly in 10 women, and subsequently categorised each test phase as early follicular (days 1-7), mid-follicular (days 7-12), mid-cycle (days 12-18), mid-luteal (days 18-21) and late-luteal (days 21-32). Quadriceps MVIF and handgrip strength

were 11.5% and 11.7% higher during the mid-cycle compared to the luteal phase, however this study was also limited by determining menstrual phase by counting days from menstruation, which is not accurate in predicting cycle phase (Wideman et al., 2013). In a similar study, Phillips et al. (1996) studied adductor pollicis MVIF in both trained (n=10) and untrained (n = 12) women by taking repeated measurements across one to three menstrual cycles and showed that MVIF increased during the follicular phase and declined after ovulation. These studies relied on periodic testing sessions, such as weekly intervals, rather than at specific, pre-determined time points, resulting in heterogeneity within-phases and an inability to determine whether the correct phase was used. This may have also resulted in some participants being included in some time points and not others, which could influence outcomes if it is not a fully-balanced, within-subject design, with all participants completing all conditions. Phillips et al. (1996) attempted to correct for this by analysing adductor pollicis MVIF in the days surrounding the LH peak relative to individuals' mean MVIF values, and showed that MVIF was significantly reduced by ~7% following the LH peak, however this was calculated using the estimated LH peak days for many participants which is not accurate due to within-participant variability in cycle length (Fehring et al., 2006).

These results were supported by Bambaeichi et al. (2004), who studied isometric and isokinetic force of the knee extensors and knee flexors in eight women, across five stages of the menstrual cycle. Menstrual cycle phase had no effect on isokinetic peak torque of the knee extensors at 1.05 rad·s^{-1}, knee flexors at 3.14 rad·s^{-1} or MVIF of the knee extensors. However, knee flexor MVIF and peak torque at 1.05 rad·s^{-1} were significantly higher at ovulation compared to the mid-follicular and mid-luteal phases and knee extensor peak torque at 3.14 rad·s^{-1} was highest during ovulation and the mid-luteal phase. This evidence suggests that maximal force production is typically greatest during the ovulatory and luteal phases, when oestrogen concentrations are highest. In contrast to these results, Tenan et al. (2016) showed that MVIF of the knee extensors was similar in the early-follicular, late-follicular, ovulatory and late-luteal phases, yet was ~23% lower in the mid-luteal phase. The authors suggested that force was inversely related to progesterone concentrations, although no hormonal analyses were conducted to confirm this. Ekenros et al. (2013) observed the opposite effects, with improved knee extensor strength (+4.2%) in the luteal phase compared to the follicular phase, although this was probably due to a learning effect, as all participants completed the luteal phase testing session after the follicular phase and similar learning effects were observed in OC-using participants.

These studies have identified differing effects of reproductive hormones, with some studies showing that muscle strength is improved at stages of the menstrual cycle when oestradiol

concentrations are highest (Phillips et al., 1996; Sarwar et al., 1996; Bambaechi et al., 2004), others showing improved performance when oestradiol concentrations are lowest (Davies et al., 1991) or medium concentrations (Wirth and Lohman, 1982) and some studies showing performance is inversely related to progesterone concentrations (Tenan et al., 2016). Within the studies that show an effect of menstrual cycle phase on indices of muscle strength, the effects appear to occur at different time points of the menstrual cycle.

These studies are also limited by methodological flaws, such as a lack of hormonal analysis to confirm phases (Bambaeichi et al., 2004; Davies et al., 1991b; Sarwar et al., 1996; Tenan et al., 2016; Wirth & Lohman, 1982), learning effects (Ekenros et al., 2013), poorly defined phases (Davies et al., 1991; Phillips et al., 1996; Sarwar et al., 1996; Wirth & Lohman, 1982) and a lack of standardisation of diet and activity, which can alter reproductive hormone concentrations or affect muscle strength (Bambaeichi et al., 2004; Davies et al., 1991; Ekenros et al., 2013; Kraemer et al., 1995; Phillips et al., 1996; Reichman et al., 1993; Sarwar et al., 1996; Tenan et al., 2016; Wirth & Lohman, 1982).

2.4.3.2. Studies that have shown that muscle force does not change across the menstrual cycle

In contrast to previously discussed research, numerous studies have shown that muscle strength is not changeable across the menstrual cycle. The most commonly studied measure of muscle strength is isokinetic peak torque of the knee extensors (Abt et al., 2007; DiBrezzo, Fort, & Brown, 1991; Fridén, Hirschberg, & Saartok, 2003; Gür, 1997; Hertel et al., 2006; Lebrun, McKenzie, Prior, & Taunton, 1995) and flexors (Abt et al., 2007; DiBrezzo et al., 1991; Gür, 1997; Hertel et al., 2006; Lebrun et al., 1995). Other measures that have been shown to be unaffected by menstrual cycle phase are: quadriceps MVIF (Higgs & Robertson, 1981; Janse de Jonge, Boot, Thom, Ruell, & Thompson, 2001; Montgomery & Shultz, 2010) and electrically evoked contractions (Janse de Jonge et al., 2001), handgrip strength (Ekenros et al., 2013; Fridén et al., 2003; Higgs & Robertson, 1981; Janse de Jonge et al., 2001; Nicolay, Kenney, & Lucki, 2008), FDI muscle MVIF (Elliott, Cable, Reilly, & Diver, 2003) and maximal isometric lifting strength (Birch & Reilly, 2002).

As with studies that have shown changes in muscle strength across the menstrual cycle, many studies are limited by methodological flaws including studies that have not confirmed ovulation (Davies et al., 1991b; Gür, 1997; Hertel et al., 2006; Nicolay et al., 2008), not assayed hormones to confirm phase (Nicolay et al., 2008), and not controlled for diet and exercise (Abt et al., 2007; DiBrezzo et al., 1991; Fridén et al., 2003; Gür, 1997; Hertel et al., 2006; Janse de Jonge et al., 2001; Nicolay et al., 2008).

2.4.3.3. Summary of highest-quality evidence

As there are many studies with significant methodological flaws, it is important to take this into consideration when reviewing the evidence. The highest-quality evidence are studies which have used well-defined phases of the menstrual cycle based upon physiological set-points, and confirmed using hormonal analyses. Based upon these criteria, only 10 studies provide an acceptable level of evidence (Abt et al., 2007; Ekenros et al., 2013; Elliott et al., 2003; Fridén et al., 2003; Giacomoni, Bernard, Gavarry, Altare, & Falgairette, 2000; Gür, 1997; Hertel et al., 2006; Janse de Jonge et al., 2001; Kubo et al., 2009; Lebrun et al., 1995). Of these studies, only one has shown that muscle force production was different across the menstrual cycle (Ekenros et al., 2013), and this was possibly due to a learning effect, as all test sessions were completed in the same order and a similar result was apparent in the OC group. The majority of these studies have used small numbers of participants (≤ 10; Abt et al., 2007; Elliott et al., 2003; Fridén et al., 2003; Hertel et al., 2006; Kubo et al., 2009) and have not controlled diet and exercise in the lead-in to test sessions (Abt et al., 2007; Ekenros et al., 2013; Fridén et al., 2003; Gür, 1997; Hertel et al., 2006; Janse de Jonge et al., 2001; Lebrun et al., 1995). Whilst the majority of high-quality evidence suggests no effects of reproductive hormones throughout the menstrual cycle on muscle force production, these studies are still limited by not addressing all pertinent methodological issues, so further well-designed research addressing these issues is required to confirm these results.

Table 2.3. Summary of studies assessing muscle function across phases of the menstrual cycle.

Authors	Participants	Time points	Determination of phase	Muscle function measures	Hormonal analysis	Main results
Abt et al. (2007)	10 EU women	EF (D3), OV (24-36h post LH surge), ML (7D post OV)	Days from menses (EF), Days post-LH surge (OV, ML)	Isokinetic quadriceps and hamstring average peak torque at $60°s^{-1}$ (5 reps) and $180°s^{-1}$ (10 reps)	E_2, P_4	↔ between phases for all muscle function measures
Bambaeichi et al. (2004)	8 EU women	EF (D1-4), MF (D7-9), OV (24-36h post-LH surge), ML (D19-21), LL (D25-27)	Days from menses (EF, MF, ML, LL), post-LH surge (OV)	Isokinetic knee extensor and flexor MVIF and peak torque at 1.05 and $3.14rad·s^{-1}$	None	↑ knee flexor MVIF and peak torque at $1.05rad·s^{-1}$ in OV vs. MF and ML ↑ knee extensor peak torque at $3.14rad·s^{-1}$ in OV vs. ML ↔ knee extensor MVIF and peak torque at $1.05rad·s^{-1}$, or knee flexor peak torque at $3.14rad·s^{-1}$
Davies (1991)	12 EU women	EF (D1-4), LF (D12-14), ML (D19-21)	Count-forward from menses	Standing long jump and handgrip strength	None	↑ handgrip strength in EF phase vs. LF and ML phase ↑ jump distance in EF phase vs. LF phase, but not different to ML phase
Dibrezzo et al. (1991)	21 EU women	EF (D1), OV (D13-14), ML (D23-34)	Count-forward from menses	Isokinetic knee extensor and flexor peak torque at 60, 180 and $120°s^{-1}$ and muscle endurance (20 reps)	None	↔ between phases for all muscle function measures
Ekenros et al. (2013)	17 women (9 initially EU, 8 initially OC users), observed through one OC and one non-OC cycle	EF (D2-4), OV (24-48h post LH surge), ML (7-8D post-OV). Same protocol in OC after cessation of OC use and confirmation of OV	Days from menses (EF), Days post-LH surge (OV, ML)	Isokinetic knee extensor peak torque at $120°s^{-1}$ (5 reps), handgrip strength, One-leg hop test for distance	E_2, P_4, FSH, LH	↔ muscle function measures between OC cycle and non-OC cycle ↔ handgrip strength and one-leg hop test performance between menstrual cycle phases ↑ isokinetic peak torque in ML vs. EF phase
Elliott et al. (2003)	7 EU women	EF (D2) and ML (7 days post ovulation)	Days from menses (EF), Days post-LH surge (ML)	MVIF of the FDI muscle and	Bioavailable E_2 and T, total P_4	↔ MVIF of the FDI muscle
Friden et al. (2003)	10 EU women	EF (D3-4), OV (24-48 h post OV), ML (7 days post-OV) during two cycles	Days from menses (EF), Days post-LH surge (OV, ML)	Handgrip strength, one-leg hop test, Isokinetic knee extensor peak torque at $120°s^{-1}$ and muscle endurance (50 reps)	E_2, P_4, FSH, LH	↔ between phases for all muscle function measures

Study	Participants	Phases	Verification	Measures	Hormones	Results
Giacomonni et al. (2000)	7 EU women	EF (D1-4), MF (D7-9) and ML (D19-21)	Days from menses, confirmed with P_4	Force velocity test (4 x 8s cycle sprint), multi-jump test (5 x maximal jumps) and squat jump test (90° knee flexion, non-countermovement)	P_4	↔ between phases for all muscle function measures
Gur, (1997)	16 EU women	EF (D1-3), LF (D8-10), LP (D19-21)	Days from menses, confirmed with P_4	Knee extensor and flexor peak torque at $60°s^{-1}$ and $120°s^{-1}$ (x 4)	E_2, P_4, FSH, LH, prolactin, T, free T	↔ between phases for all muscle function measures
Hertel et al. (2006)	14 EU women	MF (D4-7), OV (OV ± 2D), ML (7-10D post-OV)	1-month monitoring period. Days from menses (MF) and predicted OV and ML based upon previous cycle	Isokinetic knee extensor and flexor at $120°s^{-1}$ (10 reps)	E3G, PdG	↔ between phases for all muscle function measures
Janse de Jonge et al. (2001)	19 EU women	EF (D1-3), LF x 2 with highest E_2 used (N.S.), ML (N.S.)	BBT used to estimate timing of phases	Quadriceps MVIF and electrically stimulated contractions. Isokinetic knee extensor and flexor peak torque at $60°s^{-1}$ and $240°s^{-1}$ (5 reps). Isokinetic endurance at $240°s^{-1}$ (60 reps). Handgrip strength	E_2, P_4, FSH, LH	↔ between phases for all muscle function measures No correlations between any hormone and any muscle function measure
Kubo et al. (2009)	8 EU women	EF (D1-3), OV(± 2 days predicted OV)	BBT used to estimated OV from 2 previous cycles and confirmed using hormones	MVIF of knee extensors and plantar flexors and resting twitch properties; peak twitch torque, time to peak torque, half-relaxation time, activation level	E_2, P_4, LH	↔ between phases for all muscle function measures
Lebrun et al. (1995)	16 trained EU women	Two successive days in EF (D3-8, actual D5.7 ± 0.5) and ML (D4-9 post OV, actual D23.3 ± 0.9).	BBT used to identify ovulation	D1: $\dot{V}O_2max$, treadmill anaerobic speed test (8 miles·h^{-1} at 20% incline until exhaustion). D2: Endurance capacity (90% $\dot{V}O_2max$), isokinetic peak torque of knee extensors and flexors at $30°s^{-1}$	E_2, P_4,	↔ isokinetic peak torque ↔ anaerobic performance and endurance performance ↑ absolute $\dot{V}O_2max$ in EF vs. ML, ↔ relative $\dot{V}O_2max$

Montgomery and Shultz (2010)	71 EU women	FP (all; D1-6), EL (n = 29; D1-3 post LH surge), ML (n = 32; D4-8 post LH surge), anovulatory (n =10)	Days from menses (FP), Days post-LH surge (EL, ML) or no OV (anovulatory)	MVIF knee extensor and flexor	E_2, P_4, T	↔ between phases for all muscle function measures
Nicolay et al. (2008)	11 EU women	EF (D4-6), LF (D11-13), ML (D20-23)	Days from menses	Handgrip strength MVIF, 20s repetition dynamic grip test, 30s static hold test	None	↔ MVIF and dynamic endurance. Static endurance (30s) lowest in the LF phase
Phillips et al. (1996)	Trained EU (n = 10), untrained EU (n = 12)	Trained measured 3x·wk^{-1}, untrained 8x over 1-3 cycles. Measured in relation to LH peak (estimated)	BBT	MVIF of the adductor pollicis	E_2 (n = 9 trained) LH (trained)	↑ MVIF over the FP: trained regression analyses = $0.62 \pm 0.24\%$·day^{-1} (P = 0.0112, n = 112), untrained = $1.05 \pm 0.51\%$·day^{-1} (P = 0.049, n = 30) Between OV and 2 days post OV, -3.07%·day^{-1} change in relative MVIF 7% ↓ in MVIF between day 11-13 No correlation between oestradiol and muscle force
Sarwar et al. (1996)	10 EU women	Tested weekly and subsequently categorised into EF (D1-7), MF(D7-12), MC (D12-18), ML (D18-21), LL (21-32)	Count-back from menses	Quadriceps MVIF, electrically evoked contractions and fatigability (40Hz for 0.25s·s^{-1} for 3 mins and handgrip strength	None	↑ Quadriceps MVIF in MC vs. all other phases (11.7% vs. LL) ↑ Handgrip strength in MC vs. all other phases (11.5% vs. LL) Relaxation time from evoked contraction ↓ at MC vs. EF, ML and LL ↔ time to half-relaxation ↑ fatigability in MC vs. ML and LL
Tenan et al. (2016)	9 EU women	EF, LF, OV, ML, LL phases	BBT 1-2 months prior and during cycle	Knee extensor MVIF and muscle endurance (25% MVIF hold)	None	↓ MVIF in ML vs. LF, OV and LL MVIF ↔ in EF ,LF, OV and LL ↔ Muscle endurance
Wirth and Lohman (1982)	10 EU women	FP (6-10), ML (18-24)	Days from menses	Handgrip strength MVC and endurance time at 50%	None	↑ MVC in the FP vs LP

Anovulatory, AO; Basal body temperature, BBT; Oestradiol, E_2; Ostrone-3-gluconaride. E3G; Early follicular phase, EF; Early luteal phase, EL; Eumenorrheic, EU; Follicular phase, FP; First Dorsal Interosseus, FDI; Follicle stimulating hormone, FSH; Late luteal phase, LL; Luteinising hormone, LH; Mid luteal phase, ML; Maximal voluntary isometric force, MVIF; Not specified, N.S; Ovulatory phase, OV; Progesterone, P_4; Pregnandiol-3-gluconaride, Pdg; Testosterone, T

2.4.4. Oral contraceptives

Several studies have assessed muscle force production across an OC cycle (Table 2.4). These studies have assessed various indices of muscle strength at differing time points of an OC cycle, typically using combined (Ekenros et al., 2013; Elliott, Cable, & Reilly, 2005; Giacomoni et al., 2000; Rechichi & Dawson, 2009; Sarwar et al., 1996) or unspecified (Nicolay et al., 2008; Phillips et al., 1996; Wirth & Lohman, 1982) preparations. Varying indices of muscle force production have been assessed including isokinetic peak torque of the knee extensors (Ekenros et al., 2013; Elliott et al., 2005) and flexors (Elliott et al., 2005), electrically evoked (Sarwar et al., 1996) and MVIF of the quadriceps (Elliott et al., 2005), MVIF of the FDI muscle (Elliott et al., 2005) and adductor pollicis (Phillips et al., 1996), handgrip strength (Ekenros et al., 2013; Nicolay et al., 2008; Sarwar et al., 1996; Wirth & Lohman, 1982) and dynamic muscle strength, such as jumping and hopping (Ekenros et al., 2013; Giacomoni et al., 2000a; Rechichi & Dawson, 2009).

With one exception (Rechichi & Dawson, 2009), measures of muscle force production have been shown to be unchanged between different phases of the OC cycle. Rechichi and Dawson (2009) showed that while measures of peak power, repeated sprint performance and countermovement jump were unaffected, there were differences in reactive strength from a drop jump between different phases of an OC cycle. Reactive strength from a 30cm drop was lowest during the late PFI compared to the early PFI and pill consumption phase, whereas reactive strength from a 45cm drop was highest during pill consumption compared to both PFI phases. Rechichi and Dawson (2009) studied the early and late PFI as these represent different reproductive hormone profiles. In the early PFI, exogenous hormones may still be present in small concentrations whilst endogenous oestrogens remain low, whereas endogenous oestrogens are higher in the late PFI and exogenous hormones are fully cleared from the system (Carol et al., 1992; Rechichi & Dawson, 2009; van Heusden & Fauser, 1999). This is the only study to date to compare different stages within the PFI, and suggests that there may be an effect of these different stages. It is currently unclear however, as these data appear conflicting with reactive strength from a 30 cm and 45 cm drop performance being greatest at different stages of the OC cycle.

The majority of muscle strength measures have not shown any differences between phases of OC use, however all studies to date have either not reported the preparations of OCs used by participants, or have used mixed OC preparations. The use of different OC preparations will deliver different types and concentrations of synthetic hormones, in addition to resulting in differing concentrations of endogenous oestradiol concentrations (Elliott-Sale et al., 2013). This results in a higher inter-individual variability within participant groups, which may cause

type II errors. Further studies are needed that use the same preparation of OC in order to reduce between-participant variability and minimise the risk of false-negative outcomes.

The timing of test sessions in relation to pill consumption is also important, because peak concentrations of synthetic hormones are observed in the circulation approximately 1 h following OC consumption, and are reduced by 50% within 3 h (Kuhnz et al., 1992b). To date, only one study has standardised the timing of pill consumption in relation to muscle force assessments (Elliott et al., 2005), conducting performance tests 1 h post OC consumption. Future studies should standardise the timing of pill consumption in relation to testing, as any potential effects of synthetic hormones will not be discernible if the circulating concentrations are poorly controlled.

Table 2.4. Summary of studies assessing muscle function across phases of an oral contraceptive cycle.

Authors	Participants	Time points	Preparations	Muscle function measures	Hormonal analysis	Main results
Ekenros et al. (2013)	17 OC users. 8 started study using OCs and 9 started OCs during study and were tested in 2nd pill packet	D7-8 PC, D14-15 PC, D2-4 PFI	Combined, low dose (20-35µg EO) with varying progestins	Isokinetic knee extensor peak torque at 120°s^{-1} (5 reps), handgrip strength, One-leg hop test for distance	E$_2$, P$_4$, FSH, LH	No overall difference in muscle function measures between OC cycle and non-OC cycle $\leftrightarrow$ muscle function measures between different phases of OC cycle
Elliott et al. (2005)	14 OC users	D7 PC, D14 PC, D5 PFI	Combined, low dose (30-35µg EO) with varying progestins	MVIF of the FDI, quadriceps and hamstrings and isokinetic peak torque at 1.04 rad·s^{-1}, 2.09 rad·s^{-1} and 4.19 rad·s^{-1}	E$_2$, P$_4$	$\leftrightarrow$ any muscle function measure between phases of the OC cycle
Giacomoni et al. (2000)	10 OC users	D1-4 of PFI, D1-2 PC, D12-14 PC	Combined, low dose (20-30µg EO) with varying progestins	Force velocity test (4 x 8s cycle sprint), multi-jump test (5 x maximal jumps) and squat jump test (90° knee flexion, non-countermovement)		$\leftrightarrow$ between phases for all muscle function measures
Nicolay et al. (2008)	8 OC users	D4-6 PFI, D4-6 PC, D13-16 PC	Not specified	Handgrip strength MVIF, 20-s repetition dynamic grip test, 30-s static hold test	None	$\leftrightarrow$ between phases for all muscle function measures
Phillips et al. (1996)	5 trained OC users	3x·wk^{-1} for ~6 months	Not specified	MVIF of the adductor pollicis muscle	E$_2$	$\leftrightarrow$ MVIF across an OC cycle No correlation between oestradiol and muscle force
Rechichi and Dawson (2010)	10 OC users	D13-17 PC, D2-3 PFI, D6-7 PFI	Combined, low dose (30-35µg EO) with varying progestins	Countermovement jump (without arm swing), drop jumps from 30 and 45cm, 10s bike power test, repeated sprint test (5 x 6s with 24s recovery)	E$_2$, P$_4$	$\leftrightarrow$ Countermovement jump, peak power and repeated sprint performance Reactive strength from 30cm $\downarrow$ at D6-7 PFI vs. other phases Reactive strength from 45cm $\uparrow$ at PC vs. both PFI phases

Sarwar et al. (1996)	10 OC users	Tested weekly during two cycles	Combined, low dose (20-35µg EO) with varying progestins	Quadriceps MVIF, electrically evoked contractions and fatiguability (40Hz for $0.25s \cdot s^{-1}$ for 3 mins and handgrip strength	None	↔ between phases for all muscle function measures
Wirth and Lohman (1982)	16 OC users (9 supplementing with Vit B)	D6-10 and D18-24	Not specified, but 8 separate brands	Handgrip strength MVC and endurance time at 50%	None	↔ between time points for all muscle function measures

Oestradiol, E_2; Ethinyl oestradiol, EO; First dorsal interosseus, FDI; Follicle stimulating hormone, FSH; Luteinizing hormone, LH; Maximal voluntary isometric force, MVIF; Oral contraceptive, OC; Progesterone, P_4; Pill consumption, PC; Pill free interval, PFI

2.4.5. Energy availability and muscle force production

Low energy availability has been speculated to reduce muscle strength (Mountjoy et al., 2014), although there is currently little evidence available to support this (De Souza et al., 2014). It is important to make the distinction between long-term reductions in energy availability, which impairs muscle size, structure and strength (Zibellini et al., 2016), in comparison to short-term reductions in energy availability, where there is currently little available evidence. Whilst research has assessed the response of muscle function to short-term energy restriction prior to competition in weight category sports (Degoutte et al., 2006; Filaire, Maso, Degoutte, Jouanel, & Lac, 2001; Fogelholm, Koskinen, Laakso, Rankinen, & Ruokonen, 1993; Oöpik et al., 1996; Serfass, Stull, Alexander, & Ewing, 1984; Timpmann, Oöpik, Pääsuke, Medijainen, & Ereline, 2008; Viitasalo, Kyröläinen, Bosco, & Alen, 1987; Webster, Rutt, & Weltman, 1990), these athletes restrict fluid intake, resulting in dehydration, which is known to have a detrimental effect on muscle strength (Savoie, Kenefick, Ely, Cheuvront, & Goulet, 2015) Therefore, this research will not be discussed as it is not applicable to populations with a low energy availability, that do not intentionally engage in dehydration practices to meet a specific weight criterion, such as athletes with disordered eating practices or athletes that inadvertently fail to match their energy intake to their demands (Nattiv et al., 2007).

2.4.5.1. Muscle function in severe energy restriction and refeeding

Lopes et al. (1982) showed that muscle contractile properties were different in malnourished patients (n = 10), that had recently lost weight due to severe gastric disorders, compared to controls (Lopes et al., 1982). Under electrical stimulation, the adductor pollicis muscle was more fatiguable, had a slower relaxation rate and an altered force-frequency relationship, whereby a greater proportion of maximal force was produced at low frequency (10 Hz) stimulations. This altered force-frequency relationship most likely occurred due to the slowed relaxation rate, whereby a tetanus occurred at lower frequency stimulations. There was a negative correlation between body mass lost and relaxation rate, suggesting that the degree of energy restriction may affect muscle contractile properties. Four of these patients underwent total parenteral nutrition as part of their treatment, which resulted in an improvement in relaxation rate to levels seen in controls and improved fatiguability (Lopes et al., 1982). Increased fatiguability, slowed relaxation rate and similar alterations to the force-frequency relationship were also observed in six anorexia nervosa patients (63% of ideal body weight) admitted to a medical unit, which was improved to 'normal' values by an 8 week re-feeding programme, coinciding with a 19% increase in body mass (Russell et al., 1983). After the re-feeding programme, patients were still only 73% of ideal body weight, suggesting that the altered muscle contractile properties after re-feeding were a result of their current nutritional, energy replete, status, rather than their body composition returning to 'normal' values. To

confirm this, a study assessing two weeks of a severely restrictive diet (400 kcal·day^{-1}), followed by two weeks of complete fasting, was conducted in obese patients that were 193% ideal body weight (Russell et al., 1983). Muscle contractile properties were similar to controls at baseline, however after 2 and 4 weeks of a severely restrictive diet, obese patients had similar muscle contractile characteristics to those seen in anorexic and malnourished patients, which was improved following a 2 week re-feeding period. These data suggest that muscle contractile properties are altered due to acute energy restriction, independent of changes in body composition, as these effects are observed in obese and underweight individuals and are reversed in response to re-feeding (Lopes et al., 1982; Russell et al., 1983; Russell et al., 1983).

It has been suggested that these changes in muscle function occur due to increases in intracellular calcium concentrations with energy restriction (Russell et al., 1983; Russell et al., 1984), which activates calcium-dependent proteases (calpains; Belcastro, Albisser, & Littlejohn, 1996). This results in immediate degradation of myofibrillar structural proteins including tropomyosin, troponin and titin (Huang & Forsberg, 1998), and causes a decentralisation of myosin filaments within the sarcomere, Z-line streaming, disturbances to the sarcoplasmic reticulum T-tubular system and a loss of sarcolemmal integrity (Belcastro, Shewchuk, & Raj, 1998; Huang & Forsberg, 1998; Murphy, 2010).

Throughout these studies, it is important to note that voluntary force was not significantly affected by energy status in the adductor pollicis muscle, however this evidence does suggest that perturbations to the muscle environment occur with short-term energy restriction. Whilst changes to the force frequency relationship and relaxation are potentially important, of more consequence are the changes to the ultrastructure of the muscle in response to short term energy restriction (Russell et al., 1984), where degradation may have more significant effects on voluntary muscle force production in muscles, especially larger muscles which have not been studied and are more relevant for athletic populations.

2.4.5.2. Short term energy restriction studies in active populations

Despite evidence that the metabolic and structural environment of muscle may be acutely affected by energy restriction, few studies have assessed the effects of short-term (≤ 2 weeks) energy restriction on muscle force production in normal weight individuals or athletic populations. Zachwieja et al., (2001) studied the effects of two weeks of moderate dietary restriction in males (n = 13) and females (n = 11), where daily energy intake was reduced by 750 kcal in the diet group (n = 16) and maintained in the control group (n = 8), with both groups undertaking 500 kcal of treadmill exercise each day. There was no change in 1

repetition maximum performance for leg press or shoulder press from pre- to post-condition in either group, suggesting minimal effects of energy restriction on strength in this population.

In a similar study, Parkes et al., (1998) compared isokinetic force of the knee extensors in women that were energy replete (100% required energy intake) or energy restricted (75% required energy intake), over a two week period, whilst undertaking ~100 min·day^{-1} of mixed-modality exercise. In the energy restricted group, eccentric average torque at 180 deg·s^{-1} was increased, while concentric peak torque at 120 deg·s^{-1} was reduced from pre- to post-diet. These data are difficult to interpret however, as the control group improved eccentric average torque at 180 deg·s^{-1} and concentric average torque at 30 deg·s^{-1}, which may have been a result of inadequate standardisation of dynamometer positioning during assessments, as indicated by the authors.

Based upon the lean body mass (51.9 ± 1.8 kg), energy intake (2398 ± 155 kcal) and exercise energy expenditure (463 ± 6 kcal) values reported, energy restricted participants in Zachwieja at al. (2001) had a mean energy availability of approximately 37.3 kcal·kgLBM·day^{-1}. In Parkes et al. (1998), the body fat percentage, as determined by skinfold callipers, was 21.8 ± 1.2 %, body mass was 65.1 ± 2.4 kg, energy intake was 1928 ± 103 kcal and exercise energy expenditure was 834 ± 142 kcal. Therefore, it can be approximated that mean energy availability was 21.5 kcal·kgLBM·day^{-1}. Whilst energy availability was not directly reported in these studies, the body composition, energy intake and exercise energy expenditure values indicated that the levels of energy availability used in these studies are not as low as those seen in some athlete groups, with several studies reporting very low values of ~11-18 kcal·kgLBM·day^{-1} (Schaal et al., 2016; Vanheest et al., 2014). Therefore, to date, studies that have assessed the effects of energy restriction on muscle strength have used relatively moderate levels of dietary restriction (Parkes et al., 1998; Zachwieja et al., 2001), which are less restrictive than that seen in some athletes (Loucks et al., 2011; Melin et al., 2015a). For example, the moderate degree of energy restriction used by Parkes et al., (1998), did not alter muscle calpain concentrations, the purported mechanism by which short-term energy restriction affects force production, and therefore may not have been severe enough to influence muscle force production. These studies (Parkes et al., 1998; Zachwieja et al., 2001) used ~100 min·day^{-1} of mixed-modality exercise, or 500 kcal·day^{-1} of treadmill running, meaning that the effects of diet-induced energy restriction and exercise-induced energy restriction have not been studied separately. This would provide further clarification of the importance of whether the method by which energy restriction is achieved affects muscle strength. Currently, very little research exists exploring the role of short term low energy

availability in active females and further research is required to explore these effects and identify whether reduced muscle strength is a negative consequence of RED-S.

2.5. Cognitive function

Cognitive function describes the ability to perceive, evaluate, store, manipulate and use information from external sources (*i.e.*, our environment) and internal sources (*i.e.*, experience, memory, concepts, thoughts), and to respond to this information (Schmitt, Benton, & Kallus, 2005). The Diagnostic and Statistical Manual for Mental Disorders (Sachdev et al., 2014) categorises cognitive functions into six domains (Figure 2.6). Performance in these domains can be affected by mood, arousal, well-being and motivation among other factors (Schmitt et al., 2005). Cognitive functions are routinely assessed in clinical populations to identify the presence of neurological disorders or cognitive impairments, but are also used in the general population to assess changes in cognition in response to a stimulus (*e.g.*, nutrient, drug, sleep, exercise) or other factors (*e.g.*, age).

In order to perform optimally, athletes are required to continually use information from external sources and accurately and repeatedly use this information to execute actions. For example, athletes may be required to adopt strategies to adjust to changing situations, encode and attend to a moving target, interpret specific proprioceptive information, or respond quickly to incoming objects (Schmit, Hausswirth, Le Meur, & Duffield, 2017). A brief summary of the cognitive domains is presented below, although language and social cognition domains are not described as these are not relevant to athletic performance.
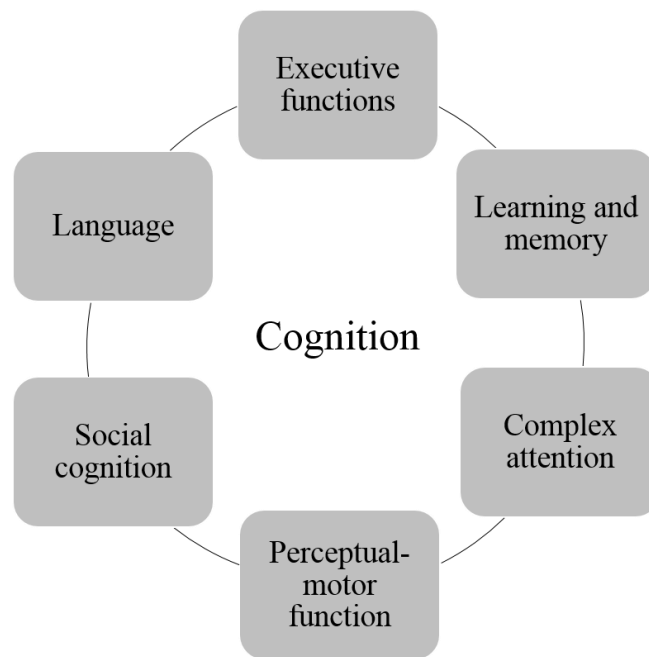
Figure 2.6. Overview of the domains of cognitive function, adapted from Sachdev et al. (2014).

2.5.1. Domains of cognitive function

2.5.1.1. Executive functions

Executive functions are cognitive processes that are required to complete complex tasks that often require an adaptive response to novel situations (Lezak, 2004; Strauss, Sherman, Spreen, & Spreen, 2006). Therefore, executive functions are important for decision making, planning and organisation, reasoning, strategic thinking and judgement (Anderson, 1998; Schmitt et al., 2005). It is often difficult to specifically assess executive function as, by definition, executive functions involve the use of other cognitive processes (Miyake et al., 2000). For example, a non-executive component, such as working memory, may be necessary for optimal performance on a test designed to assess executive function, so it is not always possible to isolate the cause for impaired performance. Miyake et al., (2000) suggested that executive function can be separated into three more basic executive processes: 1. shifting between multiple tasks (shifting), 2. monitoring relevance of incoming information and, where appropriate, replacing old information with updated information (updating) and 3. deliberately inhibiting automatic responses (inhibition).

2.5.1.2. Learning and Memory

Learning and memory describe the process of encoding, storing and retrieving information (Klein, 2015), which can be categorised into several sub-sets. Working (short-term) memory refers to the ability to store information in the short-term (seconds to 1-2 min), using this information to perform tasks (Strauss et al., 2006) and ensuring it will be available until

information is encoded into the long-term memory (Goldman-Rakic, 1992). Working memory has been described as having two 'slave' systems; the phonological loop, used to store and process auditory information, and the visual-spatial sketchpad, used to encode visual information (Baddeley & Hitch, 1974). These are controlled by the central executive, which forms strategies for utilising this information (Baddeley & Hitch, 1974). Long-term memory can be separated into explicit memory, which is the intentional and conscious recollection of previous experiences, or implicit memory, which is the sub-conscious encoding of information that can be used at a later date (Roediger, 1990).

2.5.1.3. Attention

Attention can be defined as a system of interacting components that allows the individual to filter relevant and irrelevant information, hold and manipulate mental representations, and modulate responses to stimuli (Strauss et al., 2006). Attention is comprised of sensory selection (*e.g.*, filtering, focussing), response selection (*e.g.*, response intention, active switching), attentional capacity (*e.g.*, arousal, effort) and sustained performance (*e.g.*, fatigability, vigilance; Cohen, 2014). Sustained attention (vigilance) is typically seen as the most important measure, as some attentional abnormalities are only noticed over long test periods (Strauss et al., 2006).

2.5.1.4. Perceptual-motor function

Perceptual-motor function describes the organisation, identification and interpretation of visual, auditory or somatosensory information, and the co-ordination of an appropriate motor response (Strauss et al., 2006). Sport-specific tests of perceptual-motor function have been developed that assess cognitive performance within a sporting context (McMorris & Graydon, 1997; Schwab & Memmert, 2012), however simple and choice response times to stimuli are also components of perceptual motor-functions. When interpreting results in these tests, it is important to consider whether changes in performance are resultant from alterations to information-processing speed and accuracy, or the co-ordination of an appropriate motor response (Strauss et al., 2006).

2.5.2. Cognitive function and reproductive hormones

The effects of reproductive hormones on cognitive performance have been explored using a variety of different models of reproductive functioning, including the menopause (Hogervorst, Williams, Budge, Riedel, & Jolles, 2000), HRT (LeBlanc, Janowsky, Chan, & Nelson, 2001), the menstrual cycle (Hampson, 1990), OC use (Griksiene & Ruksenas, 2011), GnRH agonist treatment (Craig et al., 2007), gender reassignment (Slabbekoorn, van Goozen, Megens, Gooren, & Cohen-Kettenis, 1999) and pregnancy (Henry & Sherwin, 2012). For athletes, the

menstrual cycle and OC use are the two most pertinent models of reproductive functioning, therefore this thesis will place a focus on studies assessing cognitive function in these models. There have been numerous studies that have assessed elements of cognitive function across the menstrual cycle or OC use, using a multitude of tests. An emphasis will be placed on studies that have measured reproductive hormones to confirm endogenous hormone profiles, as many initial studies failed to do this (Sundstrom Poromaa & Gingnell, 2014), making it possible that participants were not in the required menstrual cycle phase, affecting the interpretation of these studies (Becker et al., 2005).

The majority of research across the menstrual cycle has been conducted in 'sexually dimorphic' tasks where there are differences in performance between genders. Males typically outperform females in numeric/mathematical (Feingold, 1988; Geary, 1996; Gouchie & Kimura, 1991) and spatial awareness tasks (Andreano & Cahill, 2009; Collins & Kimura, 1997; Gouchie & Kimura, 1991; Linn & Petersen, 1985; Voyer, Voyer, & Bryden, 1995), while women typically outperform men in tasks of verbal fluency, perceptual speed, fine motor skills and verbal memory (Basso, Harrington, Matson, & Lowery, 2000; Bleecker, Bolla-Wilson, Agnew, & Meyers, 1988; Feingold, 1988; Hall & Kimura, 1995; Mann, Sasanuma, Sakuma, & Masaki, 1990). The magnitude of these differences is relatively small, although differences are more apparent with increasing task complexity (Hyde & Linn, 1988; Linn & Petersen, 1985). Further evidence that cognitive performance is modifiable by reproductive hormones is provided by studying individuals undergoing gender reassignment, where transitioning from male to female and using oestrogen and anti-androgen therapy improved verbal memory (Miles, Green, Sanders, & Hines, 1998), while transitioning from female to male and using androgen therapy, improved performance in visuospatial tasks (Slabbekoorn et al., 1999; Van Goozen, Cohen-Kettenis, Gooren, Frijda, & Van de Poll, 1994, 1995). Therefore, tests of spatial awareness, verbal memory and verbal fluency have been a focus of studies assessing cognitive performance across the menstrual cycle and OC cycle.

2.5.3. Cognitive function and the menstrual cycle

2.5.3.1. Mental rotation test

The mental rotation test, an aspect of perceptual-motor function, has been identified as having the largest gender disparity of the spatial awareness tasks (Andreano & Cahill, 2009), and therefore has been the most commonly studied test across the menstrual cycle. Several studies have shown that mental rotation test performance is greatest in the early follicular phase, when oestrogen concentrations are lowest (Courvoisier et al., 2013; Hampson, Levy-Cooperman, & Korman, 2014; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Güntürkün, 2000; Maki, Rich, & Shayna Rosenbaum, 2002). These studies have assessed two time points (early

follicular and mid-luteal phase) of the menstrual cycle, so are unable to properly elucidate the effects of reproductive hormones on performance (Hausmann et al., 2000; Maki et al., 2002), used a cross-sectional design, where between-group differences may not be attributable to hormone-related effects (Hampson et al., 2014) or measured performance daily for 8 weeks, which may be confounded by practice and ceiling effects (Courvoisier et al., 2013). Nevertheless, these studies have typically shown a negative correlation between oestradiol concentrations and mental rotation performance (Hampson et al., 2014; Hausmann et al., 2000; Maki et al., 2002).

In contrast, several studies have shown no effect of menstrual cycle phase on mental rotation test performance (Dietrich et al., 2001; Epting & Overman, 1998; Gordon & Lee, 1993; Griksiene & Ruksenas, 2011; Mordecai et al., 2008; Schöning et al., 2007). These studies have either used small (< 10) sample sizes (Dietrich et al., 2001; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Güntürkün, 2000), used a composite score for visuospatial tests, so the effects on mental rotation are not clear (Gordon & Lee, 1993), used tests with a low difficulty level, (Epting & Overman, 1998), which are less likely to be able to detect differences due to ceiling effects (Hyde & Linn, 1988; Linn & Petersen, 1985), measured performance at two time points of the menstrual cycle, so are unable to properly elucidate the effects reproductive hormones performance (Dietrich et al., 2001; Epting & Overman, 1998; Mordecai, Rubin, & Maki, 2008; Schöning et al., 2007), or not controlled diet and activity prior to test sessions (Dietrich et al., 2001; Epting & Overman, 1998; Gordon & Lee, 1993; Griksiene & Ruksenas, 2011; Mordecai et al., 2008; Schöning et al., 2007), which can influence cognitive performance (Chang et al., 2012; Cooper, Bandelow, & Nevill, 2011). A meta-analysis showed a non-significant standardised mean difference in error rate of 1.61 (CI – 0.35 to 3.57) between early follicular phase and mid-luteal phase mental rotation performance, suggesting that spatial awareness is not affected by reproductive hormone concentrations, although only 6 studies were eligible for this analysis as studies that did not include measurement of hormone concentrations were excluded (Sundstrom Poromaa & Gingnell, 2014). All studies that have observed an effect showed that performance was greatest during the early follicular phase, although further research is required that addresses the previously identified limitations, in addition to measuring mental rotation performance at several phases of the menstrual cycle, in order to more fully elucidate the effects of reproductive hormones.

2.5.3.2. Verbal fluency and verbal memory
Some studies have shown that verbal fluency (Maki et al., 2002; Solís-Ortiz & Corsi-Cabrera, 2008) and verbal memory (Maki et al., 2002; Rosenberg & Park, 2002) are improved at phases

of the menstrual cycle where oestradiol concentrations are highest. These studies had small sample sizes (< 10) (Rosenberg & Park, 2002; Solís-Ortiz & Corsi-Cabrera, 2008), or have only tested during the early follicular and mid-luteal phases so are unable to differentiate the effects of oestradiol and progesterone (Maki et al., 2002; Mordecai et al., 2008). Furthermore, a number of studies have shown that there were no differences in verbal fluency (Griksiene & Ruksenas, 2011; Hampson, 1990; Mordecai et al., 2008) or verbal memory performance between menstrual cycle phases (Hatta & Nagaya, 2009; Jacobs & D'Esposito, 2011; Phillips & Sherwin, 1992). These studies are also limited by only measuring performance in two phases, restricting the ability to differentiate the effects of oestrogen and progesterone (Hampson, 1990; Hatta & Nagaya, 2009; Jacobs & D'Esposito, 2011; Mordecai et al., 2008; Phillips & Sherwin, 1992), and not controlling pre-test diet and activity (Griksiene & Ruksenas, 2011; Hampson, 1990; Hatta & Nagaya, 2009; Mordecai et al., 2008; Phillips & Sherwin, 1992), which affects cognitive performance. Further research is required that addresses these limitations and measures performance at several menstrual cycle phases to differentiate the effects of oestrogen and progesterone on verbal fluency and verbal memory.

2.5.3.3. Executive functions

Few studies have assessed executive function across the menstrual cycle, however studies assessing performance in shifting (Farrar, Neill, Scally, Tuffnell, & Marshall, 2015; Symonds, Gallagher, Thompson, & Young, 2004) or updating (Broverman et al., 1981; Farrar et al., 2015; Hampson, 1990; Man, MacMillan, Scott, & Young, 1999; Solís-Ortiz & Corsi-Cabrera, 2008) elements of executive function have not shown differences between menstrual cycle phases. There is a small sex difference in Stroop test performance, with women generally having shorter response times and smaller interference effects than men (Mekarski, Cutmore, & Suboski, 1996; Van der Elst, Van Boxtel, Van Breukelen, & Jolles, 2006), therefore tests of inhibition of automated responses, such as the Stroop test, have been the most studied across the menstrual cycle. Stroop test performance has been shown to be highest in the early follicular phase (Upadhayay & Guragain, 2014), highest in the mid luteal phase (Lord & Taylor, 1991), or not to vary across the menstrual cycle phases (Keenan, Stern, Janowsky, & Pedersen, 1992; Keenan, Lindamer, & Jong, 1995; Resnick, Perry, Parry, Mostofi, & Udell, 1998). These studies did not, however, measure hormone concentrations to confirm menstrual cycle phase so may not accurately reflect the desired reproductive hormone concentrations (Fehring et al., 2006). To date, only one study has measured hormone concentrations when measuring Stroop test performance across the menstrual cycle (Hatta & Nagaya, 2009). Hatta & Nagaya (2009) showed that accuracy in the Stroop test was 13.6% higher in the mid-luteal phase compared to the early follicular phase, suggesting a beneficial effect of oestrogen on Stroop test performance. All studies assessing Stroop performance across the menstrual cycle

to date have only assessed two phases (early follicular and mid-luteal), so further research is required assessing additional phases to differentiate the effects of oestrogen and progesterone.

2.5.3.4. Attention

Several studies have shown no difference in sustained attention tasks (Griksiene & Ruksenas, 2009; Keenan, Lindamer, & Jong, 1995a; Resnick, Perry, Parry, Mostofi, & Udell, 1998) between the early follicular and mid luteal phases, while others have shown a slower response time (Matthews & Ryan, 1994) and poorer accuracy in the mid luteal phase (Pletzer, Harris, & Ortner, 2017). In contrast, Solís-Ortiz et al., (2008) showed that response time, number of correct responses, number of errors and number of omissions in a continuous performance task were all poorer in the ovulatory phase compared to the early luteal phase. The majority of these studies have not measured hormone concentrations to confirm cycle phases and are, therefore, unable to accurately determine reproductive hormone concentrations, so these results are questionable. Only two studies have measured hormone concentrations and assessed attention over several phases of the menstrual cycle (Brötzner, Klimesch, & Kerschbaum, 2015; Griksiene & Ruksenas, 2011), Brötzner et al., (2015) showed that visuospatial attention response time and accuracy did not vary between phases, however as this test contained a spatial component, which has previously been shown to be affected by menstrual cycle phase (Maki et al., 2002), this may confound the interpretation of the attentional component of the test. Griksiene and Ruksenas (2009) also showed that performance in the Anfimov table task (modified letter cancellation test) was not different between menstrual cycle phases, however this short (1 min) test of visual scanning does not assess other aspects of attention such as sustained attention/vigilance. Further research is required on additional attentional tasks, that measures hormone concentrations to correctly identify phases, and that use tests that isolate attentional components from other cognitive domains as much as possible.

2.5.3.5. Perceptual-motor performance

Producing a quick and accurate response to visual and auditory stimuli and generating an appropriate motor response is vital to performance in tests that assess a variety of cognitive domains. Few studies have attempted to assess response time to simple stimuli that require minimal loading of other cognitive processes (*e.g.*, visuospatial, working memory, attention) across the menstrual cycle. Simple response time has been shown to be slowest in the mid-luteal phase (Matthews & Ryan, 1994; Slade & Jenner, 1980), while others have shown the slowest response times occur in the early follicular phase (Kumar, Mufti, & Kisan, 2013; Šimić & Ravlić, 2013) and some research shows no differences between menstrual cycle phases (Griksiene & Ruksenas, 2009; Loucks & Thompson, 1968; Morgan & Rapkin, 2002).

It has been suggested that reproductive hormone effects on renin-angiotensin and aldosterone result in retention of sodium and body water during the luteal phase which may elongate axonal conduction time and negatively affect neurotransmitter function (Babyminakshi et al., 2006; Hirshoren et al., 2002; Walpurger et al., 2004), however current evidence on the effects of this on response time across the menstrual cycle are unclear. Studies assessing response time across the menstrual cycle have either not measured reproductive hormone concentrations to confirm menstrual cycle phases, not confirmed ovulation, not controlled diet and exercise, or only assessed simple response time. Further, well controlled research is required to assess response time to simple and complex stimuli using well-defined phases of the menstrual cycle.

2.5.4. Cognitive function and oral contraceptives

Cross-sectional studies have shown that OC users have superior immediate and delayed memory and attention (Gogos, 2013), improved spatial ability (Wharton et al., 2008) and poorer verbal fluency performance compared to non-users (Griksiene & Ruksenas, 2011), with the poorest verbal fluency performance observed in those using androgenic OCs (Griksiene & Ruksenas, 2011). The importance of OC androgenicity was further shown by Wharton et al., (2008), where spatial ability was greatest in women who used the most androgenic OCs (second generation), while third generation OC users' spatial ability was also superior to fourth generation OC users. Furthermore, third generation OC users had significantly longer response times in a mental rotation task compared to non-OC users (Wharton et al., 2008). It is unclear, however, if these differences are a result of brain structural changes due to long-term use of these OCs, differences in concentrations of circulating endogenous and exogenous hormones at the time of testing with OC use, or a combination of these factors.

Several studies have been conducted assessing within-OC cycle changes in cognitive function to assess the importance of synthetic hormone concentrations on cognitive function (Gordon & Lee, 1993; Griksiene & Ruksenas, 2009, 2011; Moody, 1997; Mordecai et al., 2008; Rosenberg & Park, 2002). As with studies of the menstrual cycle, sexually dimorphic tasks, especially mental rotation tests, verbal fluency and verbal memory, have been the primary focus of OC-based research due to the existing link with reproductive hormones, although there is a comparative lack of research on OC use compared to the menstrual cycle.

2.5.4.1. Mental rotation

For mental rotation tasks, studies to date have shown no effect of OC phase on spatial ability (Gordon & Lee, 1993; Griksiene & Ruksenas, 2011; Moody, 1997; Mordecai et al., 2008).

These studies have used mixed-pill groups; increasing inter-individual variability (Elliott-Sale et al., 2013), which may be especially important in cognitive function due to the influence of different androgenicity pills on cognition (Gogos, 2013; Wharton et al., 2008). This may increase the chance of false-negative results and further research is required that assesses mental rotation on pill consumption days and during the PFI, using an homogenous group, in order to identify if OC phase influences performance.

2.5.4.2. Verbal fluency and verbal memory

Previous studies show there was no effect of OC use on verbal fluency (Gordon & Lee, 1993; Griksiene & Ruksenas, 2011; Mordecai et al., 2008) or verbal memory (Rosenberg & Park, 2002), although one study showed that memory performance was greater in the active treatment of OC use compared to the PFI (Mordecai et al., 2008). Mordecai et al., (2008) measured memory performance on the California Verbal Learning Test in the PFI and late pill-consumption phases, suggesting that synthetic hormone administration may be beneficial for memory. Further research should be conducted on verbal memory due to the conflicting results, although it is also necessary to assess performance in the early pill consumption phase, in order to examine the effects of exogenous hormones on verbal memory, as the concentration of exogenous synthetic hormones is known to increase throughout pill consumption days (Carol et al., 1992).

2.5.4.3. Oral contraceptives and other cognitive domains

Similar to studies of the menstrual cycle, other domains of cognitive function have not been well-studied across phases of OC consumption. Current research suggests that auditory and verbal response time (Griksiene & Ruksenas, 2009), attention (Griksiene & Ruksenas, 2009; Mordecai et al., 2008), visual memory (Griksiene & Ruksenas, 2009; Mordecai et al., 2008) and complex co-ordination (Baisden & Gibson, 1975) are not different between OC phases, while executive functions, including the Stroop test, have not been studied. Further research is required exploring Stroop test performance, in addition to other cognitive domains, improving previous research by assessing during the PFI, early and late pill consumption phases, and using a homogeneous OC group to reduce inter-individual variation and the likelihood of type II errors occurring (Elliott-Sale et al., 2013).

2.5.5. Energy availability and cognitive function

The RED-S model outlines that aspects related to cognitive function such as judgement, concentration and co-ordination are impaired with low energy availability (Mountjoy et al., 2014), although beyond self-reported decrements in these areas in athletes with low energy

availability (Ackerman et al., 2018), there is little available research to evidence this in exercising females. The majority of evidence of the effects of energy restriction on cognitive performance concerns clinical populations, overweight individuals on a weight loss programme, or military personnel, which will be briefly discussed below. Studies assessing the cognitive response to rapid pre-competition weight loss in weight category sports will not be discussed as the concomitant dehydration in these athletes (Artioli et al., 2010) confounds interpretation due to the negative effects of dehydration on cognitive performance (Grandjean & Grandjean, 2007). As with the research into muscle force production, the studies in this areas have not measured energy availability and therefore energy status is typically described as energy restriction, proportionate to the amount of energy required for energy balance.

2.5.5.1. Clinical models of energy restriction

Sundgot-Borgen & Torstveit (2004) showed that 13.5% of 1620 elite athletes had sub-clinical or clinical eating disorders, with values of up to 42% reported in aesthetic sports, highlighting the importance of clinical models of energy restriction for this population. Anorexia nervosa patients have been shown to have poorer response time (Green, Elliman, Wakeling, & Rogers, 1996), psychomotor performance (Green et al., 1996), and working memory (Green et al., 1996; Mathias & Kent, 1998) than controls and an altered brain structure with reduced hippocampal volume (~8% Connan et al., 2006). Despite a 12 week dietary intervention which increased weight, improved affect and reduced psychopathology scores, there were no improvements in cognitive performance in anorexia nervosa patients, suggesting that the impairments were potentially due to a brain structural adaptation rather than short-term dietary effect (Green, Elliman, Wakeling, & Rogers, 1996). Furthermore, bulimia nervosa patients have been shown to have poorer attention and perception than controls with the poorest cognitive performance observed in those with pathologically high beta-hydroxybutyrate levels, indicative of metabolic starvation. While cognitive function has been shown to be poorer than controls in some studies (Green et al., 1996; Mathias & Kent, 1998), others have shown no effect (Connan et al., 2006). It is difficult to identify whether chronic energy restriction affects cognitive performance in these individuals as clinical eating disorders are typically co-morbid with depression and an altered mood state which may impact cognition independently of energy related factors (Austin, Mitchell, & Goodwin, 2001).

2.5.5.2. Psychological aspects of dieting

Dieting has been associated with increased depression and reduced self-esteem (Ackard, Croll, & Kearney-Cooke, 2002; French, Perry, Leon, & Fulkerson, 1995), which are known to negatively impact cognitive function (Austin, Mitchell, & Goodwin, 2001). A number of studies have shown that women currently dieting to lose weight had poorer performance on

tests of attention (Green, Rogers, Elliman, & Gatenby, 1994), response time (Green et al., 1994), and memory (Green & Rogers, 1998; Green et al., 1994), regardless of whether they actually lose weight (Green & Rogers, 1995). This suggests that cognitive impairments may be a result of the psychological responses to the process of being on a diet, rather than alterations to energy availability. Furthermore, it has been demonstrated that these impairments are primarily a result of impaired working memory capacity (Green et al., 1994; Shaw & Tiggemann, 2004). Kemps and Tiggemann (2005) showed that dieting status did not affect the phonological loop or visuospatial sketchpad (i.e. the slave systems of the working memory model), but did affect performance when these were combined (i.e. the central executive), indicating that current dieting status selectively impacts central executive functioning, which could have wide-ranging effects on cognitive performance (Kemps & Tiggemann, 2005). Impaired cognitive performance in dieters has been associated with preoccupying thoughts concerning hunger (Green, Elliman, & Rogers, 1997) and body-shape self-esteem (Green & Rogers, 1998; Vreugdenburg, Bryan, & Kemps, 2003) and has been suggested to preferentially consume working memory capacity which is important for many aspects of cognition (Green & Rogers, 1998).

Importantly, cognitive impairments have mostly been observed in unsupervised dieters where individuals are attempting to lose weight in a self-selected manner. Weight loss regimens involving prescribed dietary intakes appear to have minimal effects of cognitive performance (Bryan & Tiggemann, 2001; Kretsch, Green, Fong, Elliman, & Johnson, 1997; Martin et al., 2007), with some studies even showing improved memory performance (+24.4%) after a 12 week diet consuming 50% of energy required for weight maintenance (Kretsch et al., 1997). This suggests that following a regimented, prescribed diet reduces pre-occupying thoughts that consume working memory capacity and is associated with more favourable effects on cognitive function. This is supported by evidence showing that unsupported dieting is associated with a greater degree of eating disordered psychopathology and body dissatisfaction (Juda, Campbell, & Crawford, 2004) and an unsupported weight loss (n = 16) group had poorer vigilance, executive functioning and memory performance compared to supported dieters (n = 14) and controls (n = 16) one week into a weight loss programme, despite similar changes in body mass and composition (Green, Elliman, & Kretsch, 2005). This evidence suggests that it is important that dieters should have a supported environment to reduce the likelihood of cognition being affected and that prescribed diets may limit the effects seen in energy restriction studies, which should be considered in future research.

2.5.5.3. Short-term energy restriction

The effects of short-term energy restriction on cognitive function have not been widely researched, with few studies assessing the cognitive responses to < 1 week of energy restriction outside weight category sports. Several studies have assessed the omission of one or several meals on cognitive function and shown inconsistent results, with no domain of cognitive function consistently affected by short-term (2-24 h) periods of fasting (Benau, Orloff, Janke, Serpell, & Timko, 2014). The effects of several days' energy restriction have received even less attention in the literature.

Lieberman et al., (2008) compared two days of a very low calorie diet (183 kcal·day^{-1} energy intake) to energy balanced conditions consisting of either carbohydrate or carbohydrate and fat diets (~2820 kcal·day^{-1} energy intake), with all conditions including 2 h·day^{-1} of low intensity exercise (40-45% heart rate reserve). Consuming the calorie restricted diet had no effect on vigilance, response time, memory and reasoning skills, and did not influence participants' self-reported mood, suggesting that even very restrictive diets do not impact cognition. However, only a small proportion of the study population (2/27) were female, thereby limiting the applicability of these findings to female athletes.

In a further study, twenty-three participants (male n = 17, female n = 6) completed ~4h·day^{-1} of exercise at 40-65% V̇O$_2$peak and consumed either an energy balanced (3935 ± 769 kcal·day^{-1} energy intake), or calorie-restricted (266 ± 61 kcal·day^{-1} energy intake) diet (Lieberman et al., 2017). Grammatical reasoning and choice reaction time were improved in the calorie restricted condition compared to the energy balanced group, while performance in tests of vigilance and working memory were unaffected. The altered performance was associated with reduced vigour and increased tension, fatigue and total mood disturbance and reduced interstitial glucose concentrations in the calorie restricted condition. This is supported by animal models of starvation where aspects of cognitive function are improved in rats on a severely restrictive diet (40% required energy intake), purportedly as an evolutionary adaptation to increase food acquisition when necessary (Martin et al., 2008). The improved cognition in rats was only observed in the female rats, with no effect apparent in the males. There was also a potential effect of gender on cognitive function in the Lieberman et al., (2017) study, whereby females (n = 6) performed better in tests of working memory, grammatical reasoning and vigilance in the calorie restricted condition, which was not apparent in males (n = 17). This evidence suggests that females may be more responsive to short-term energy restriction induced changes in cognitive function and therefore that reproductive hormones may influence this response. Research should be conducted to assess this in a larger population of women, as previous studies have used only two (Lieberman et al., 2008) and six (Lieberman

et al., 2017) female participants and focussed on male participants. Furthermore, the role of reproductive hormones should be explored by comparing the cognitive response to energy restriction in different models of reproductive functioning, such as eumenorrheic females and OC users, which have different endogenous and exogenous reproductive hormone milieus.

Changes in cognitive performance were only observed when a greater exercise duration was used (Lieberman et al., 2017), which resulted in a greater deviation from energy balance (-3681 kcal·day^{-1} compared to -2138 kcal·day^{-1} in the earlier study), despite similar dietary energy intakes in the calorie restricted conditions. The severity of the dietary restriction combined with the longer duration of exercise would have resulted in a negative energy availability, which is only representative of extreme situations such as military training, and does not represent practices of athletes (Melin et al., 2015a). Therefore, further research is required which assesses an energy availability more representative of the practices of athletes. Furthermore, exercise is known to have effects on cognitive function independent of its effects on energy balance (Tomporowski, 2003), therefore future studies should assess a non-exercise control group and a dietary restriction-only group to differentiate between the effects of energy availability and the potential confounding effects of exercise on cognitive performance.

In these studies (Lieberman et al., 2008, 2017), participants were blinded to their condition with the use of non-nutritive food and low calorie gels, which may reduce the ecological validity of these experiments as in real-world environments, individuals are aware of whether they are restricting dietary intake. The participants were unable to differentiate between energy restricted and energy balanced conditions, and therefore they may not have experienced pre-occupying thoughts concerning hunger, which have previously been show to impact cognitive functions (Green et al., 1997). Future research is required that uses an ecologically valid model.

2.6. Bone metabolism

The Triad and RED-S models outline that bone metabolism, structure and function are compromised by low energy availability and/or menstrual dysfunction (De Souza et al., 2014; Mountjoy et al., 2014). A focus will be placed on the effects of reproductive hormones during the menstrual cycle and OC cycle on bone metabolism in this chapter, as the effects of energy restriction have previously been discussed in Section 2.3 as part of the Triad and this thesis does not examine the bone response to low energy availability.

2.6.1. Bone metabolism overview

Bone is a dynamic tissue that is constantly remodelled through resorption and formation processes. The rates of bone resorption and formation are typically coupled so that bone mass and structure are maintained, although uncoupling of bone turnover can occur for many reasons (Delaisse, 2014). Imbalances in bone turnover, in favour of resorption over formation, results in reduced bone mass, compromised bone structure and can lead to an increased risk of fractures (Vasikaran, 2008). Biochemical markers of bone metabolism can be used to identify changes in bone metabolic processes and can be used as a predictor of fracture risk (Ivaska, Gerdhem, Väänänen, Akesson, & Obrant, 2010; Vasikaran et al., 2011) and provide an acute indicator of the efficacy of interventions on bone health (Chaki et al., 2000; Lu et al., 2012; Vasikaran, 2008). Bone metabolic markers are principally used to assess bone formation through osteoblast activity (*e.g.*, BAP, P1NP, osteocalcin) or bone resorption through osteoclast activity (*e.g.*, β-CTX, NTX, DPD) and have been used to assess the bone metabolic environment (Vasikaran et al., 2011). Over 20 different markers have been used to assess bone metabolism, which limits the ability of researchers to compare results across studies (Vasikaran et al., 2011). As such, the International Osteoporosis Foundation (IOF), National Bone Health Alliance and International Federation of Clinical Chemistry and Laboratory Bone Marker Working Standards Group have recommended a consistent approach regarding the use of bone markers, recommending that P1NP and β-CTX are used to measure bone formation and resorption, controlling for factors such as circadian rhythm, fasting status and exercise to reduce pre-analytical variability (Szulc et al., 2017; Vasikaran et al., 2011; Lee & Vasikaran, 2012).

2.6.2. Bone metabolism and reproductive hormones

Reproductive hormones are important regulators of bone turnover, with oestrogen receptors expressed by osteoblasts (Eriksen et al., 1988; Komm et al., 1988), osteoclasts (Martin-Millan et al., 2010; Oursler, Pederson, Fitzpatrick, Riggs, & Spelsberg, 1994) and osteocytes (Kondoh et al., 2014). Progesterone nuclear receptors present in osteoblasts (MacNamara & Loughrey, 1998) and osteoclasts (Pensler, Radosevich, Higbee, & Langman, 1990). Oestrogen primarily acts by supressing osteoclastogenesis, thereby inhibiting bone resorption (Chen, Wang, & Huang, 2009), whilst progesterone is a bone-trophic that increases osteoblast proliferation (Scheven, Damen, Hamilton, Verhaar, & Duursma, 1992). Accordingly, naturally occurring states with low oestrogen and progesterone concentrations, such as the menopause and hypothalamic amenorrhea, are associated with increased bone turnover, with greater relative increases in bone resorption than formation, resulting in reduced BMD (Garnero, Sornay-Rendu, Claustrat, & Delmas, 2000; Miller & Klibanski, 1999). Oral

contraception downregulates endogenous reproductive hormones and provides synthetic oestrogens and progestins in small doses, which may influence bone health. Oral contraceptive use in adolescence has been shown to impair the achievement of optimal peak bone mass (Cibula, Skrenkova, Hill, & Stepan, 2012; Hartard et al., 2007; Pikkarainen, Lehtonen-Veromaa, Möttönen, Kautiainen, & Viikari, 2008; Polatti, Perotti, Filippa, Gallina, & Nappi, 1995), although these effects are not apparent after peak bone mass is achieved, and several studies show that OC use improved BMD compared to non-users in women after the age of 30 years (Gambacciani, Ciaponi, Cappagli, Benussi, & Genazzani, 2000; Gambacciani et al., 2006; Nappi, Bifulco, Tommaselli, Gargano, & Di Carlo, 2012). The mechanisms surrounding the effects of OC use on bone health remain to be elucidated (Trémollieres, 2013), therefore further research is required that explores the bone metabolic response of OC users compared to eumenorrheic counterparts.

2.6.3. The menstrual cycle

The menstrual cycle has been used as a model to study the effects of reproductive hormones on bone metabolism for nearly 40 years. Initial studies focused on calcium-regulating hormones and showed lower parathyroid hormone (PTH) concentrations and higher ionised calcium concentrations in the luteal phase of the menstrual cycle, suggesting oestrogen inhibits PTH-induced bone resorption (Pitkin, Reynolds, Williams, & Hargis, 1978). This variation in PTH concentrations across the menstrual cycle was not, however, shown in other studies (Gray, McAdoo, Hatley, Lester, & Thierry, 1982). Other calcium regulating hormones, such as 1,25-dihydroxyvitamin D_3, have been shown to vary across the menstrual cycle (Gray et al., 1982) and to remain unchanged (Baran et al., 1980; Muse et al., 1986; Nielsen et al., 1990) by different studies. Differences in outcomes between these studies are most likely due to differences in the phases used to measure calcium-regulating hormones and non-standardisation of diet and exercise pre-measurement, which can affect hormone concentrations (Kraemer et al., 1995; Nakamura, Aizawa, Imai, Kono, & Mezaki, 2011). Since 1990, biochemical markers of bone metabolism in urine, serum and plasma have been used as a more direct indicator of the effects of the menstrual cycle on bone formation and resorption processes than calcium-regulating hormones, although a wide range of bone metabolic markers have been used with different assays, experimental protocols and participants, so the effects of cyclical fluctuations in reproductive hormone concentrations on bone metabolism are impossible to interpret (Table 2.5).

2.6.3.1. Bone formation

The most studied marker of bone formation across the menstrual cycle is osteocalcin, a non-collagenous protein in the bone matrix synthesised by osteoblasts during bone formation.

Whilst several studies have shown no changes in osteocalcin across the menstrual cycle (Chiu et al., 1999; Gorai et al., 1998; López Moreno, González, Campino, Salvatierra, & Croxatto, 1992; Schlemmer et al., 1993), other studies have shown the highest concentrations during the ovulatory phase (Lee et al., 2012) and luteal phase (Gass, Kagan, Kohles, & Martens, 2008; Nielsen, Brixen, Bouillon, & Mosekilde, 1990). Nielsen et al. (1990) showed that osteocalcin was cross-correlated with oestradiol with a time lag of 7 days, suggesting a possible delayed stimulatory effect of oestradiol on osteoblastic activity. These studies, however, measured total osteocalcin, which includes the under-carboxylated, non-bone-specific fraction, rather than measurements of carboxylated osteocalcin only, which is specific to bone metabolic processes (Razny et al., 2017), meaning that these studies cannot be used to accurately assess bone formation.

P1NP and P1CP are peptides cleaved from osteoblast-synthesised procollagen molecules, with circulating concentrations reflecting the rate of collagen type 1 production and are, therefore, also used as a marker of bone formation. Gorai et al. (1998) showed the highest concentrations of P1CP in the early follicular phase compared to the mid-luteal phase, whereas Zitterman et al. (2000) showed that P1CP concentrations were highest 3-days post-ovulation and were lowest in the early follicular phase. Another study showed no differences between phases (Schlemmer et al., 1993) and it is unclear why these studies produced opposing results, although it may be due to Zitterman et al. (2000) using a younger population (25 ± 3 years) compared to the other studies (mean ~33 years; Gorai et al., 1998; Schlemmer et al., 1993), or differences in ethnicity between study populations, which both influence bone turnover markers (Costa et al., 2013; Jorgetti, dos Reis, & Ott, 2014). Furthermore, Zitterman et al. (2000) measured bone turnover markers at time points of the menstrual cycle based upon the timing of ovulation in previous cycles, which has been shown to be inaccurate (Gordon et al., 1986). Only two studies have assessed the concentrations of P1NP across the menstrual cycle in healthy women (Gass et al., 2008; Liakou et al., 2016), despite this being the recommended marker of bone formation by the IOF (Vasikaran et al., 2011; Szulc et al., 2017). These studies showed that P1NP concentrations were 6.4% (Gass et al., 2008) and 11.4% (Liakou et al., 2016) higher in the luteal phase compared to the follicular phase, although there are methodological issues that need to be considered. Liakou et al. (2016) used a population aged 30-45 years (mean 33.6 ± 4.5 years), which is older than the majority of athletes; important because age significantly influences bone metabolism, including a 31% reduction in P1NP concentrations between individuals aged 20-30 years and 30-39 years (Jenkins et al., 2013). Furthermore, Gass et al. (2008) did not report the assays used to measure P1NP, which are needed to determine appropriateness of the assay according to IOF guidelines (Szulc et al., 2017). Neither study (Gass et al., 2008; Liakou et al., 2016) controlled for exercise prior to

sample collection, which is likely to impact the bone marker response (Weiler, Keen, & Wolman, 2012). Further well-controlled studies are needed to clarify the changes in P1NP across the menstrual cycle.

While its use is recommended by the IOF (Vasikaran et al., 2011), P1NP is not specific to bone, as circulating concentrations can also reflect skin and connective tissue metabolism (Szulc et al., 2017). In contrast, BAP concentrations are reflective of bone-metabolic processes only and represent bone mineralisation, a separate component of osteoblast function compared to the collagen turnover marker P1NP. Therefore, measurements of both P1NP and BAP provide a more comprehensive indication of bone metabolic processes. Across the menstrual cycle, BAP was shown to have a negative cross-correlation with oestradiol concentrations measured 6 days earlier (Chiu et al., 1999), and BAP concentrations have also been shown to rise throughout the follicular phase (Gass et al., 2008; Gorai et al., 1998) and be higher in the luteal phase compared to the follicular phase (Nielsen et al., 1990). In contrast, other studies have shown that concentrations of BAP (Niethammer, Körner, Schmidmayr, Luppa, & Seifert-Klauss, 2015) and serum alkaline phosphatase (Schlemmer et al., 1993), albeit a less specific marker of bone formation (Pagani, Francucci, & Moro, 2005), do not vary across the menstrual cycle. There are currently conflicting data for BAP, so further research is required to evaluate whether cyclical changes in reproductive hormones across the menstrual cycle influences BAP concentrations.

2.6.3.1. Bone resorption

The response of bone resorption markers across the menstrual cycle is variable between studies. The medium in which formation markers are measured (urine or serum) may influence the responses across the menstrual cycle and, therefore, these will be discussed separately. The pyridium cross-links (PYD and DPD) are present in bone and are released during bone resorption and measured in urine; they have been shown to be elevated when oestrogen concentrations are lower during the follicular phase (Chiu et al., 1999; Gorai et al., 1998; Zittermann et al., 2000) compared to ovulatory and luteal phases, although others have shown no difference between phases (Niethammer et al., 2015; Schlemmer et al., 1993). In one study, DPD was negatively cross-correlated with oestradiol at a time lag of 6 and 8 days and was also negatively correlated with progesterone concurrently and at 2, 4 and 6 days earlier, suggesting that higher levels of oestradiol and progesterone inhibited bone resorption in a delayed manner (Chiu et al., 1999), although urinary measurements were taken after the first morning void which increases sample variability (Vesper, 2005). Hydroxyproline (HP) is also released into the bloodstream during bone resorption and has been shown to be in greater concentrations during the follicular phase (Gorai et al., 1998), although others have shown no

such effects (Schlemmer et al., 1993). These data should be interpreted with caution, however, as circulating HP concentrations are not specific to bone metabolic processes (Pagani et al., 2005). Urinary NTX has also shown inconsistent findings, with Gorai et al., (1995) showing higher NTX concentrations in the late follicular phase compared to the early follicular phase, whereas Gass et al. (2008) showed that NTX was higher in the early and mid-follicular phases compared to the mid and late luteal phases. Yet, others have shown no changes across the menstrual cycle (Abrahamsen, Stilgren, Rettmer, Bonnevie-Nielsen, & Beck-Nielsen, 2003; Shimizu et al., 2009). The above studies have used urinary measures of bone resorption, which may not be appropriate to examine changes across the menstrual cycle, as these values are corrected for creatinine excretion, which is increased by 8-20% in the luteal phase compared to the follicular and ovulatory phases (Davison & Noble, 1981; Paaby, Møller-Petersen, Larsen, & Raffn, 1987; Phipps, Duncan, Merz, & Kurzer, 1998). Therefore, any changes observed between phases of the menstrual cycle using urinary resorption markers may be a result of differences in renal creatinine clearance rather than the rate of bone resorption, meaning that serum markers of bone resorption are more appropriate to use in these studies.

The bone resorption marker recommended by the IOF, serum β-CTX, has been studied on several occasions and has consistently been shown to be 9-13% higher in the follicular phase compared to the luteal phase (Gass et al., 2008; Liakou et al., 2016; Mozzanega et al., 2013; Niethammer et al., 2015). However, these studies have either not reported the analytical procedures used to measure β-CTX (Gass et al., 2008; Niethammer et al., 2015), which limits the ability to interpret values (Szulc et al., 2017), used participants aged 30-45 years (Liakou et al., 2016) or > 40 years (Niethammer et al., 2015), affecting comparisons to younger individuals (Jenkins et al., 2013) or not clearly defined menstrual cycle phases used for measurements (Niethammer et al., 2015), limiting the ability to identify the time point of the menstrual cycle in which samples were collected. Furthermore, these studies have not followed recommended protocols for bone marker measurements such as collecting samples at time points outside the recommended (7.30am - 10.30am; Szulc et al., 2017) time of day (Niethammer et al., 2015), did not state whether samples were collected after an overnight fast (Mozzanega et al., 2013; Niethammer et al., 2015), and provided no indication of whether exercise was limited in the 24 h prior to measurements (Gass et al., 2008; Liakou et al., 2016; Mozzanega et al., 2013; Niethammer et al., 2015), except avoiding recruiting 'excessive exercisers' (Gass et al., 2008). Further research is required that studies serum β-CTX whilst controlling these measures to reduce pre-analytical variability.

2.6.4. Oral contraceptive use

Bone formation markers such as P1NP (Wreje et al., 2000), osteocalcin (Karlsson, Eden, & von Schoultz, 1992; Paoletti et al., 2004) and BAP (Garnero, Sornay-Rendu, & Delmas, 1995; Paoletti et al., 2004; Rome et al., 2004) are reduced with OC use of between 3 and 12 months and cross-sectional studies have also shown lower concentrations of P1NP (Glover et al., 2009), P1CP (Garnero, Sornay-Rendu, & Delmas, 1995) and osteocalcin (Garnero et al., 1995; Ott et al., 2001) in OC users. However, other prospective studies have shown no changes in osteocalcin (Gargano et al., 2008; Nappi et al., 2003; Nappi et al., 2005) with 1 years OC use and increases in osteocalcin and BAP (Endrikat et al., 2004a) after 3 years use.

Evidence suggests that the dose of EO has no effect on the bone metabolic response (Endrikat et al., 2004a; Gargano et al., 2008; Nappi et al., 2003), and where similar EO concentrations are used with different progestins there are also no differences between pill types (Nappi et al., 2005). Across studies, second generation LNG-containing OCs have increased (Endrikat et al., 2004a) and decreased (Rome et al., 2004) bone formation markers, with third generation Desogestrel (Wreje et al., 2000) and fourth generation Drospirenone (Paoletti et al., 2004) showing reductions in bone formation markers, although there is not enough evidence to attribute this to the type of progestin. The duration of OC use may, however, affect the response, as no changes in bone metabolic markers were evident after 12 months, but became apparent after 2 and 3 years (Endrikat et al., 2004a).

For bone resorption, prospective studies have shown that carboxy-terminal telopeptide of type 1 collagen (ICTP; Wreje et al., 2000) DPD (Gargano et al., 2008; Nappi et al., 2003; Nappi et al., 2005; Paoletti et al., 2004) PYD (Gargano et al., 2008; Nappi et al., 2003; Nappi et al., 2005; Paoletti et al., 2004; Rome et al., 2004) and NTX (Endrikat et al., 2004a) concentrations were reduced with OC use. In cross-sectional studies, DPD (Garnero et al., 1995) PYD (Garnero et al., 1995) and NTX (Garnero et al., 1995; Ott et al., 2001) were also lower in OC users compared to eumenorrheic women, although Schultz et al. (2012) showed that ITCP concentrations were higher in OC users compared to eumenorrheic controls. In general, OC use has been shown to reduce both bone formation and bone resorption, resulting in an overall reduction in bone turnover, which is associated with a reduction in BMD (Scholes et al., 2010), although this has also been associated with a lower fracture risk (Dombrowski, Jacob, Hadji, & Kostev, 2017; Michaëlsson, Baron, Farahmand, Persson, & Ljunghall, 1999).

2.6.4.1. Bone metabolism markers across a pill cycle

While the long-term effects of OC use have been frequently studied, the changes in bone metabolic markers throughout an OC cycle have only been studied on three occasions (Jürimäe,

Vaiksaar, Mäestu, Purge, & Jürimäe, 2011; Wreje et al., 2000; Zittermann et al., 2002). Bone marker concentrations are frequently assessed in OC users (Endrikat et al., 2004b; Nappi et al., 2003), and it is important that any variability in bone markers across an OC cycle is well defined in order to prevent type I and II errors when measures are taken at different phases of an OC cycle.

Wreje et al. (2000) showed no difference in P1NP and ICTP concentrations measured at 4 time points of an OC cycle, however this was measured after only 2 months of OC use, which may not be representative of typical OC use (Foidart et al., 2000) and in women with chronic posterior pelvic pain, where collagen metabolism may not be representative of the general population (Kristiansson, Svärdsudd, & von Schoultz, 1996). A further study (Jürimäe et al., 2011) also showed no change in the bone markers osteocalcin and ICTP between two time points of the OC cycle. These time points, however, are reported as the follicular phase (day 8 ± 3) and luteal phase (day 20 ± 2) which, if the first day of the PFI is classified as the beginning of the follicular phase, means that samples on each time point could be either pill consumption or pill withdrawal days, so it is difficult to categorise these effects, limiting the ability to interpret these data.

Zitterman et al. (2002) measured bone markers in 12 women across a pill cycle and showed that 24 h urinary β-CTX was 26% and 27% lower during early (day 3-5) and late (day 17-19) pill consumption compared to the PFI, and 2 h fasting DPD concentrations were 19% lower on pill consumption days, although this did not reach statistical significance. The use of creatinine-corrected β-CTX and DPD measurements should be interpreted with caution, since OC use increases creatinine clearance (Brändle, Gottwald, Melzer, & Sieberth, 1992) and, therefore, any differences between pill consumption and omission days may not be solely reflective of changes in bone resorption. Nevertheless, this reduction in bone resorption markers was paralleled by a significant reduction in renal calcium and phosphorous excretion at these time points which may reflect changes in bone metabolism. Although oestrogen is known to inhibit bone resorption (Chen et al., 2009), the elevated levels of bone resorption markers during pill consumption occurred when endogenous oestradiol was 39-52% lower than in the PFI. This suggests that the exogenous, synthetic, EO or progestin components of OCs may modulate bone resorption across an OC cycle as circulating EO and progestins are elevated during pill consumption (Carol et al., 1992) and activate ERs in a similar manner to endogenous oestrogen (Rabe, Bohlmann, Rehberger-Schneider, & Prifti, 2000). Despite changes in bone resorption, there was no effect on P1CP, which remained stable throughout the pill cycle, although this study used a mixed-pill group that can increase variability of endogenous hormones (Elliott-Sale et al., 2013) and, furthermore, the oestrogenic and

progestin content of OCs have been shown to influence bone metabolism (Paoletti et al., 2004; Rome et al., 2004), so these results may not be accurate.

To date, the bone metabolic markers recommended by the IOF, serum P1NP and β-CTX, have not been studied across the OC cycle in a healthy population. Further research is required to determine the importance of standardisation of OC phase when measuring bone metabolism. In addition to this, BAP has not currently been measured across an OC cycle and, as this represents a different fraction of bone metabolism to P1NP, this marker should also be assessed to determine whether it is affected by OC phase to provide a more comprehensive assessment of bone metabolism. Furthermore, previous research in OC users has assessed mixed-pill groups, which increased inter-individual variability, affecting reproductive hormone (Elliott-Sale et al., 2013) and bone marker (Rome et al., 2004) concentrations, so future research should use the same OC preparation in all participants.

Table 2 5. Overview of studies assessing markers of bone metabolism across a menstrual cycle.

Authors	Participants	Time points	Measures	Main results
Abrahamsen et al. (2003)	11 pre-menopausal women (and 11 post-menopause HRT users)	FP and LP (N.S)	IL-1β, IL-6, OPG, u-NTX, E$_2$	$\leftrightarrow$ E$_2$ and OPG $\uparrow$ IL-6 and IL-6β in LP $\downarrow$ u-NTX (10%) in LP u-NTX correlated to IL-β throughout and IL-6 in FP
Cidem (2013)	31 eumenorrheic women	EF (D2-5), LF (D11-13) and ML (D21-23) phases	E$_2$, P$_4$ Sclerostin, testosterone	$\leftrightarrow$ Sclerostin and testosterone between phases E$_2$ and P$_4$ as expected
Chiu et al. (1999)	20 eumenorrheic women	3 samples weekly (Monday/Wednesday/Friday) for one cycle	E$_2$, P$_4$, LH, BAP, osteocalcin, s-DPD, u-DPD,	$\uparrow$ s-DPD in FP vs. LP $\leftrightarrow$ BAP, osteocalcin and u-DPD, early FP BAP < mid FP BAP negatively correlated to E$_2$ 6 and 8 days prior and P$_4$ concurrently and 2, 4, and 6 days prior E$_2$ and P$_4$ not correlated to u-DPD or osteocalcin BAP positively correlated to s-DPD
Chiu et al. (2000)	20 eumenorrheic women	3 samples weekly (Monday/Wednesday/Friday) for one cycle	E$_2$, P$_4$, LH, IL-6, sIL-6R, PTH, s-Ca, u-Ca, BAP, osteocalcin, s-DPD, u-DPD	$\leftrightarrow$ IL-6 and sIL-6R between FP and LP IL-6 negatively correlated to E$_2$ during FP s-Ca positively correlated with IL-6, s-DPD and u-DPD $\uparrow$ PTH in FP vs. LP. PTH negatively correlated to E$_2$, s-Ca and u-Ca and positively correlated to IL-6
Gass et al. (2008)	55 eumenorrheic women	Three samples weekly (+/-) 1 day window	u-NTX, LH, FSH, E$_2$, P$_4$, osteocalcin, BAP, P1NP, β-CTX	$\uparrow$ β-CTX (9.5%) in FP vs. LP $\leftrightarrow$ u-NTX between phases $\uparrow$ osteocalcin in later LP vs LH peak $\uparrow$ BAP in late FP vs. early FP and LP $\uparrow$ P1NP in LP vs. FP, and between all sub phases $\uparrow$ u-NTX at early and mid FP vs. mid LP and late LP
Gorai et al. (1995)	9 eumenorrheic women	3 samples weekly (Monday/Wednesday/Friday) for one cycle	u-NTX, HP, LP	$\uparrow$ u-NTX, HP and LP over course of FP, $\downarrow$ throughout LP

Gorai et al. (1998)	10 eumenorrheic women	3 samples weekly (Monday/Wednesday/Friday) for one cycle	IL-6, sIL-6R, IL-1β, BAP, P1CP, osteocalcin, β-CTX, DPD, ICTP, sALP, PTH, FSH, LH,	sIL-6R ↑ in early and late FP, ↓ during early LP and ↑ in mid and late LP. ↑ BAP during mid and late FP ↓ P1CP in mid LP ↓ β-CTX and DPD in early and mid FP ↑ PYD and ICTP in early LP ↑ PTH in FP sIL-6R correlated with β-CTX, ALP E_2 correlated with osteocalcin and ALP
Iida et al. (2012)	42 eumenorrheic women	EF and OV phases (N.S)	u-DPD, BAP, s-NTX, E_2	↓ u-DPD in OV vs. menstrual phase ↑ E_2 during ovulation ↔ osteocalcin, BAP and s-NTX between phases
Lee et al. (2012)	14 eumenorrheic women	D1 of menses, FP (D3-5), OV (Day of LH kit) and ML phase	LH, FSH, E_2, P_4, osteocalcin, TRACP-5b	↑ TRACP-5b at ovulation compared to menstrual phase ↑ osteocalcin at ovulation, although not significant
Liakou et al. (2016)	14 eumenorrheic women	Every other day (+/- 1 day window)	E_2, P_4, FSH, LH, P1NP, β-CTX, sclerostin	↔ Sclerostin between phases ↑ β-CTX in FP vs. LP (mid FP > mid LP) ↓ P1NP in FP vs. LP (early and mid FP < mid LP) Negative association (r = -0.60-0.68) between peal E2 levels and % change in β-CTX in LP
Mozzanega et al. (2013)	20 eumenorrheic women	EF (D2-4), LF (D12-14), ML (D24-26), EF (D2-4)	β-CTX, RANKL, OPG, E_2, P_4, FSH, LH	↓ β-CTX in luteal phase vs. EF phase ↓ OPG in LF phase (D12-14) vs. EF phase ↔ RANKL OPG inversely related to E_2, and β-CTX during ovulatory phase No relationship between β-CTX and E_2, P_4 or RANKL
Nielsen et al. (1990)	8 eumenorrheic women	3 samples weekly (Monday/Wednesday/Friday) for one cycle	FSH, LH, E_2, P_4, BAP, osteocalcin, PTH	Osteocalcin and BAP highest in mid LP Osteocalcin positively correlated to E_2 measured ~7 days earlier ↔ PTH
Niethammer (2015)	9 participants tracked for a total of 176 cycles	FP (N.S) and LP (6-9 days post LH surge) phases	BAP, PYD, DPD, β-CTX	↑ β-CTX in FP ↔ BAP, PYD and DPD ↑ BAP in ovulatory cycles vs. anovulatory cycles
Schlemmer (1993)	15 eumenorrheic women	3 samples weekly (Monday/Wednesday/Friday) for one cycle	u-PYD, u-DPD, sITCP, osteocalcin, AP, E_2, HP, P1CP	↔ Osteocalcin, P1CP, AP, u-PYD, u-DPD, Hpy ↑ sITCP in luteal phase

Shimizu (2009)	15 eumenorrheic women	Follicular phase (D8 ± 4) and luteal phase (D25 ± 4)	OPG, RANKL, BAP, NTX, E_2	↔ BAP, NTX, OPG and RANKL
Wreje (2000)	11 eumenorrheic women with chronic posterior pelvic pain	MF (D5-8), LF (D9-12), EL (D20-23), LL (D24-27)	P1NP, ICTP	↑ P1NP in luteal phase ↔ ICTP
Zitterman et al. (2000)	10 eumenorrheic women	EF (D3), LF (3 days prior to OV), EL (3 days post Ov) and ML phase	P_4, LH, FSH, SHBG, E_1 E_2, PTH, DPD, PYD, P1CP	↑ u-PYD and u-DPD 3-days post ovulation ↑ P1CP and PTH 3 days post ovulation u-PYD and u-DPD significantly correlated to E_2

Bone-specific alkaline phosphatase, BAP; Carboxy-terminal cross-linking telopeptide of type 1 collagen, β-CTX; Deoxypyridinoline, DPD; Oestradiol, E_2; Early follicular, EF; Early luteal, EL; Follicular phase, FP; Follicle stimulating hormone, FSH; Hydroxyproline, HP; Hormone replacement therapy, HRT; carboxy-terminal telopeptide of type 1 collagen, ITCP; Interleukin, IL; Late follicular, LF; Luteinising hormone, LH; Late luteal, LL; Luteal phase, LP; Mid luteal, ML; Not specified, N.S; N-terminal telopeptide, NTX; Osteoprotegerin, OPG; Ovulatory, OV; C-terminal telopeptide of type 1 procollagen, P1CP; N-terminal telopeptide of type 1 procollagen, P1NP; Pyridinoline, PDY; Progesterone, P_4; Parathyroid hormone, PTH; Receptor-activator of nuclear factor Kappa-B ligand, RANKL; Serum, s-; Sex hormone binding globulin, SHBG; Tartrate resistant acid phosphatase, TRACP-5b; Urinary, u-

Chapter 3.0. General methods

3.1. Introduction

This chapter describes the methods used within experimental chapters (Chapters 4-10). All studies received ethical approval from the Nottingham Trent University Research (Humans) Ethics Committee or Nottingham Trent University non-invasive ethics committee and Chapters 9-10 also received ethical approval from the East Midlands NHS Research Ethics Committee.

3.2. Participants

Specific study inclusion and exclusion criteria are detailed within each chapter. All participants provided written informed consent for each experimental study prior to commencing the study and could withdraw from the study at any time.

3.3. Anthropometric measurements

3.3.1. Body mass and height

Body mass was measured in minimal clothing using calibrated electronic scales (Seca 875, UK), to the nearest 0.1 kg. Height was measured using a stadiometer (Seca 213, UK) to the nearest 0.1 cm, with participants stood upright and with their head in the Frankfort horizontal plane.

3.3.2. Body composition: International Society for the Advancement of Kinanthropometry

Body composition was measured using the International Society for the Advancement of Kinanthropometry (ISAK) restricted profile, by a level 1 qualified ISAK practitioner (Marfell-Jones, Olds, Steward & Carter, 2011). Standing height, body mass, eight skinfold sites (triceps, biceps, subscapular, iliac crest, supraspinale, abdominal, medial calf and front thigh) and five girths (arm relaxed, arm flexed and tensed, waist, gluteal and calf) were measured. Sum of skinfolds (mm), body mass index (kg·m^{-2}), waist to hip ratio and % body fat, as determined using the equation of Yuhasz (1974), were used to determine body composition.

3.3.3. Body composition: Dual-energy x-ray absorptiometry

Body composition was measured using a whole-body DXA scan (Lunar iDXA, GE Healthcare, USA). All scans were performed by a qualified DXA practitioner and calibration was conducted prior to all scans with a phantom as per the manufacturers guidelines. Participants were scanned in minimal clothing with all metal objects removed and were aligned centrally on the scanning bed. Participants were asked to arrive at the laboratory in a rested state, at least 3 h post prandial and euhydrated due to the known effects of exercise, food intake and

hydration on DXA scan results (Nana, Slater, Stewart, & Burke, 2015). Urine osmolality was checked prior to scanning and had to be < 800 mOsm·L^{-1}, otherwise participants were asked to consume water until the appropriate osmolality was achieved.

3.4. Assessment of muscle function

3.4.1. Standardisation procedures

For assessments of muscle function, participants arrived at the laboratory in a rested state having abstained from caffeine since the previous evening, as this is known to affect muscle force (Ryan et al., 2013). In the 24 h before test sessions, participants were asked to refrain from alcohol consumption (Vella & Cameron-Smith, 2010) and to avoid strenuous exercise (Millet, Martin, Lattier, & Ballay, 2003), unless the experimental design included exercise and muscle force was specifically being measured following exercise sessions. Testing was conducted at the same time of day to ensure that measurements were not affected by circadian variation (Martin, Carpentier, Guissard, van Hoecke, & Duchateau, 1999) and force production measures were always conducted in the same order. All participants were familiarised with the muscle function assessment procedures prior to the main experimental trials in order to negate learning effects.

3.4.2. Quadriceps force assessment

A 5-minute warm-up was completed on a cycle ergometer (Monark 894E, Sweden) at a self-selected intensity, which was replicated on each visit to the laboratory. Following this, participants were seated upright on a custom-built dynamometer (Plate 3.2) with the hip and knee joint angles set at 1.57 rad (90°) of flexion, with the chest and trunk secured using straps. The dynamometer dimensions were adjusted so that the popliteal fossa was resting gently against the seat edge when participants were seated with their lower-spine directly against the backrest. The immovable lever arm of the dynamometer, fitted with a strain gauge (Model no. 615 200kg, Vishay precision group, USA), was attached to the right leg of the participant using a padded ankle cuff and adjustable strapping just proximal to the lateral malleolus. Positioning of the dynamometer was recorded on the first visit to the laboratory and replicated for all subsequent visits. The force signal was amplified (x1000) and sampled at 2000Hz using an external A/D converter (Micro 1401; CED, UK) interfaced with a personal computer using Spike2 software (CED, UK). For assessment of quadriceps maximal voluntary isometric force (MVIF), participants completed two sub-maximal isometric contractions of the quadriceps, before performing three MVIF efforts, each lasting ~4 s and separated by 1 min. If the final effort produced the highest force value, further MVIF measurements were conducted until force ceased to improve with subsequent efforts. Externally focused verbal encouragement

was provided using the same script, pitch and tone in order to standardise measurements (Marchant, 2011) and participants were blinded to the force data until completion of the study. This protocol has previously been used in published work (Elliott et al., 2005) and the reliability of this technique is described in Chapter 4.

Plate 3.1. Custom-built dynamometer set-up for quadriceps muscle force assessment.

3.4.3. FDI muscle force assessment

Participants placed their right hand in warm water (40°C) until skin temperature was >38°C, as assessed by an infrared thermometer (ST-812, CEM, China), due to the known effect of muscle temperature on force production (Geurts, Sleivert, & Cheung, 2005). Following this, the hand was dried and secured to a custom-built dynamometer (Plate 3.3) with the forearm secured using adjustable Velcro straps and a reading lamp placed over the FDI muscle throughout testing to maintain skin temperature. The lateral side of the distal head of the proximal phalanx of the index finger was aligned with a force transducer, attached to a strain gauge. The thumb was secured with a strap around the shaft of the first phalanx, in a fully abducted position and the remaining fingers were restrained using a Velcro strap. An adjustable clamp, tightened to the shaft of the second metacarpal prevented upward movement of the index finger. The force signal was amplified (24 pin DIL strain gauge amplifier 846–171, Radio Spares, UK) and converted from analogue-to-digital (USB 6009 DSQ, National Instruments, UK) and then displayed on a laptop with DAQmx software (National Instruments, UK). Similar to the quadriceps assessment, participants completed two sub-maximal isometric contractions of the FDI muscle, before performing three MVIF efforts, each lasting ~4 s and separated by 1 min. If the final effort produced the highest force value, further MVIF

measurements were conducted until force ceased to improve with subsequent efforts. Externally focused verbal encouragement was provided using the same script, pitch and tone in order to standardise measurements (Marchant, 2011) and participants were blinded to the force data until completion of the study. This protocol has previously been used in published work (Elliott et al., 2005) and the reliability of this technique is described in Chapter 4.
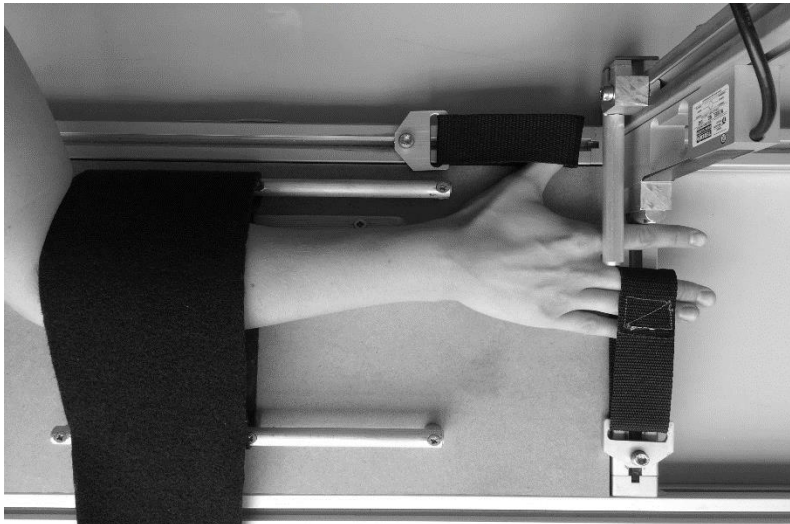
Plate 3.2. Custom-built dynamometer set-up for first dorsal interosseus (FDI) muscle force assessment.

3.4.4. Countermovement Vertical Jump with Arms (CMJA)

Participants stood upright on a jump mat (SMARTJUMP, Fusion Sport, USA) with feet shoulder-width apart and arms resting by their side. For each jump, participants were instructed to begin the downward phase of the jump, while simultaneously swinging their arms backwards, and then, without pausing at the bottom, to swing their arms forwards and jump vertically as high as possible, landing on the jump mat with legs straight (Figure 3.1). Participants were allowed to practice this movement until correctly performed during the familiarisation session and where the optimal technique was not employed during test sessions, participants were asked to repeat that effort. Vertical jump height was calculated using software analysing flight-time (Smartspeed, Fusion Sport, USA) using a tablet interface (IPAQ 112, HP, USA). After two warm-up efforts, participants performed three vertical jumps with a 1-minute rest between jumps; if the last jump was the greatest, further jumps were performed until there was no increase in jump height. Countermovement jumps with arm swing have been shown to be a very reliable measure of muscle power (Intra-class correlation [ICC] = 0.98; Markovic, Dizdar, Jukic, & Cardinale, 2004) and are representative of sport-specific movements (Gutiérrez-Dávila, Amaro, Garrido, & Javier Rojas, 2014).
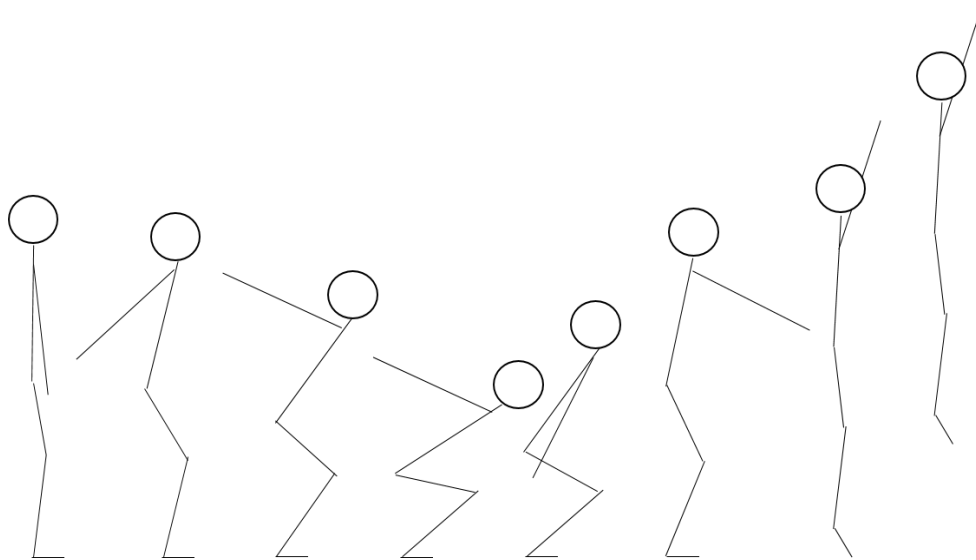
Figure 3.1. Phases of the countermovement jump.

3.5. Assessment of aerobic capacity

Maximal oxygen uptake ($\dot{V}O_2$max) was determined using a two-stage method consisting of a speed lactate test to determine running speed at lactate threshold, and a ramp test to exhaustion (Jones, 1998). Lactate threshold was determined using an incremental treadmill test with 3 minute stages beginning at 7-9 km·h^{-1} depending upon participant training history and familiarity with treadmill running. Between each stage there was a 1-minute rest period during which a capillary blood sample was taken to determine blood lactate concentration. Treadmill speed was increased by 1 km·h^{-1} per stage until blood lactate concentrations increased by 1 mmol·L^{-1} during one stage or were > 4 mmol·L^{-1}. After a 10-minute rest, participants began an exhaustive treadmill exercise test at the speed corresponding to the stage immediately before a significant increase in lactate was observed. Initial treadmill incline was 0% and was increased 1% every minute until volitional exhaustion. Breath-by-breath analysis of expired air was conducted throughout the lactate threshold and ramp test (ZAN600 CPET, nspire, USA) to determine $\dot{V}O_2$max.

3.6. Cognitive function test battery

3.6.1. Standardisation procedures and set-up

For all assessments of cognitive function, participants attended the laboratory in a rested state, having not consumed caffeine since the previous evening and refrained from alcohol consumption for at least 24 h due to the known effects on cognitive performance (McKinney, Coyle, & Verster, 2012; Nehlig, 2010). Tests were conducted at the same time of day within

each study to prevent confounding effects of circadian rhythm (Schmidt, Collette, Cajochen, & Peigneux, 2007). The cognitive test battery was performed in a quiet area, with participants seated at a desk facing a blank wall to minimise distractions. During verbal tests, the experimenter was seated directly behind the participant approximately 1 m away and participants were instructed to face away from the experimenter throughout the tests. For computer-based tasks, a laptop (Elitebook, hp, USA) was loaded with cognitive software (Sensitive Cognitive Assessment Inventory, UK), the lights were dimmed for optimal screen visibility and sound cancelling headphones were worn to prevent distractions.

A familiarisation session, consisting of a complete run-through of the cognitive tests, was conducted prior to the first experimental trial in each study to minimise learning effects. In all subsequent testing sessions, practice stimuli were provided for computer-based tasks to re-familiarise participants to the tests. A minimum of 6 practice stimuli were presented for each test, which has previously been shown to reduce practice effects and provide equivalence between assessments (Collie et al., 2003). Written instructions appeared on the screen before each cognitive task, which were reinforced with verbal instructions and participants understanding of the test was confirmed by checking that correct responses were provided during pre-test practice stimuli. For the computer-based tasks, participants were asked to get as many correct as possible, but to respond as quickly as they could in order to assess both accuracy and response time. The order the tests were completed in was standardised within each study and details are provided within each chapter.

3.6.2. Rey Auditory Verbal Learning Test (RAVLT)

The RAVLT is an oral memory test that measures immediate memory span, new learning and susceptibility to interference (Rey, 1941). A list of 15 words (List A) was read aloud (with a 1 s interval between words), for five consecutive trials (Trials 1 to 5), each followed by a free-recall test during which participants were asked to recall as many words as possible from the list in any order. The order of the presentation of the words remained fixed across trials. On completion of Trial 5, an interference list of 15 words (List B) was presented, followed by a free recall of that list. Immediately after this, participants were asked to recall List A without further presentation of these words (Trial 6). After a delay period during which the remainder of the cognitive tests were conducted (~15-25 minutes depending upon the number of tests and standardised within each study) the participants were then required to recall words from List A without hearing them again (Trial 7). Time limits were not imposed and participants were asked to inform the experimenter when they could not remember any more words.

The variables of interest were:

- Acquisition: sum of all words recalled across the 8 trials (Trials 1-7 and List B).

- Learning rate: the difference between words recalled in Trial 1 and Trial 5.

- Proactive interference: the difference between words recalled in Trial 1 and List B.

- Retroactive interference: the difference between words recalled in Trial 5 and Trial 6.

- Forgetting: the difference between Trial 7 and Trial 5.

No feedback was given regarding the number of correct responses until completion of the study. Up to six alternate words lists (Appendix 1) were used for the RAVLT during the main experimental trials. Of these, set 1 was the original list used by Rey (Lezak, 1983). The other lists were designed to match the original list for frequency of words in the English language, word length and serial position. Equivalent scores (all tests $r > 0.67$ inter-test reliability) to the original list were recorded for set 2 (Geffen, Butterworth, & Geffen, 1994), set 3 (Majdan, Sziklas, & Jones-gotman, 1996), set 4 (Crawford, Stewart, & Moore, 1989) and sets 5 and 6 (Shapiro & Harrison, 1990). The order of the sets was counterbalanced across experimental trials to prevent order effects.

3.6.3. Mental rotation test

An adapted version of the mental rotation test (Vandenberg & Kuse, 1978) was used to measure spatial awareness using a laptop. Participants were required to select, using the left and right arrow keys, which of the two three-dimensional shapes at the bottom of the screen could be rotated to match the shape in the centre of the screen. (Figure 3.2). Each trial consisted of six practice stimuli during which feedback was provided for correct and incorrect responses, followed immediately by a main trial with 50 stimuli, equally distributed between 0, 20, 40, 60 and 80 degrees of rotation relative to the central shape in a randomised order. Task difficulty is increased with greater degrees of rotation (Cooperau & Shepard, 1973) so the effects of increasing task complexity can be assessed. The variables of interest were accuracy and the response time of correct responses. For response time analysis, a minimum response time of 200 ms and a maximum response time of 20000 ms was set to remove any anticipatory or unreasonably slow response times. Tests of mental rotation have a good internal consistency (Kuder-Richardson 20 = 0.88 and Cronbachs $\alpha = 0.91$; Vandenberg & Kuse, 1978; Cassie, Vigneau & Bors, 2009) and test-retest reliability ($r = 0.83$; Vandenberg & Kuse, 1978).
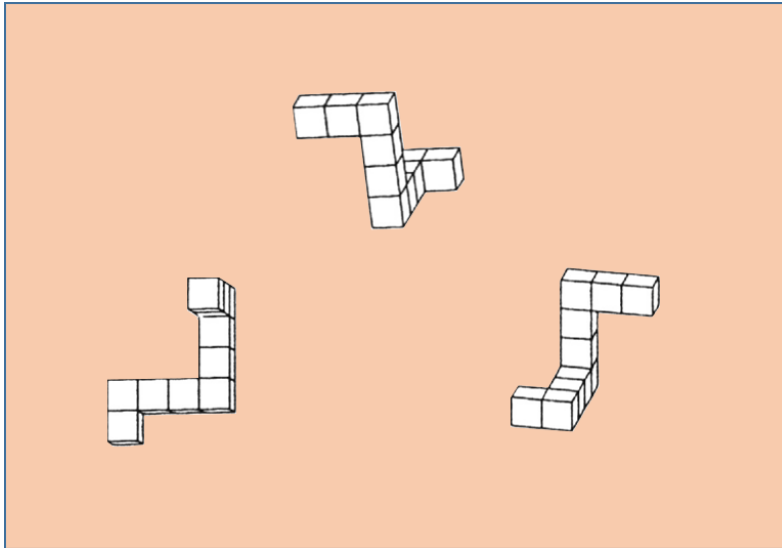
Figure 3. 2. Example stimuli within the mental rotation test.

3.6.4. Verbal fluency test

The verbal fluency test (Benton, Hamsher, & Sivan, 1989), is an oral assessment of a participant's ability to produce words given certain restrictions. In this test, participants were given a letter of the alphabet and were asked to say as many words as possible that begin with this letter in one minute, excluding capitalised words (i.e. proper nouns) or variations of the same word (*e.g.,* frame and framing). In each assessment participants were given three different letters, with a 30-second break between letters, and the total number of acceptable words said within the time limit was recorded. Phenomic fluency (*e.g.,* letters) was chosen rather than category fluency (*e.g.,* animals, colours) as the alternate forms are more comparable across repeated measurements (Strauss et al., 2006). The letter combinations used were FAS, CFL and BHR; FAS and CFL have been shown to have a high equivalence level (Cohen & Stanczak, 2000; Lacy et al., 2007; Troyer, 2000) with correlations between 0.85 and 0.94 and scores on BHR also correlate well (0.83) to FAS (Delis, Kramer, Kaplan, & Holdnack, 2004). The order of letter combinations was counterbalanced across trials to prevent order effects.

3.6.5. Visual search test

The Visual Search test is a computer-based test assessing response time and simple visuo-motor speed consisting of two difficulty levels (Figure 3.3). In the simple level, 21 stimuli were presented and participants were required to press the space bar as quickly as possible when a green triangle was presented on a black background. In the complex level, green dots were randomly distributed across the screen, which were redrawn every 250 ms to induce the effects of a flickering background and act as a distractor. The outline of a triangle was

progressively drawn on the background in green dots, with the density of the dots increasing over time. The participants were required to press the space bar as soon as they identified the triangle for a total of 21 stimuli. In both test levels, the location of the triangle stimulus was random and the variables of interest were accuracy and the response time of correct responses. For response time analysis, a minimum response time of 300 ms and a maximum response time of 1500 ms (simple level) and 10000 ms (complex level) was set to remove any anticipatory or unreasonably slow response times. The test has previously been shown to be reliable for both the simple and complex response times ($r = > 0.80$) levels (Bandelow, van der Wardt, Madden, & Hogervorst, 2011).
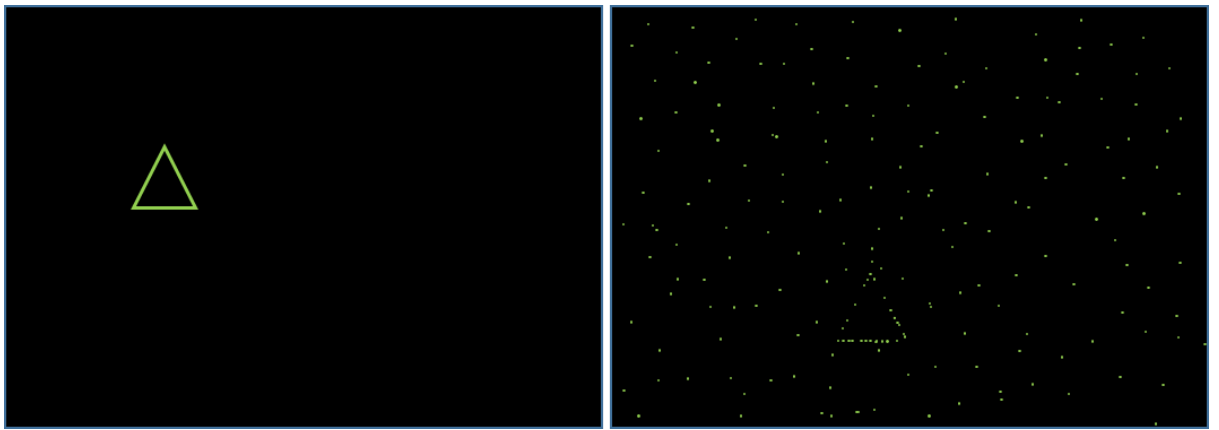
Figure 3.3. Example screenshot of baseline (left) and complex (right) levels of the visual search test.

3.6.6. Stroop-colour test

The Stroop-colour test (Stroop, 1935), is a test of executive function/inhibition that measures the ability to suppress automated responses (Strauss et al., 2006). In the simple level, one of three words (RED, BLUE or GREEN) was written in white font in the centre of the screen, with a matching word and non-matching word either side, also in white font (Figure 3.4). Using the arrow keys, participants selected the word that matched the middle word as quickly as possible for a total of 20 stimuli. In the complex level, participants were provided with a word in the centre of the screen (*e.g.,* GREEN), written in a different colour font (*e.g.,* blue font). This time, the participant was asked to choose the word corresponding to the font colour of the central word, rather than the written text, as quickly as possible for a total of 40 stimuli. The variables of interest were response time of correct responses and accuracy. For response time analysis, a minimum response time of 250 ms and a maximum response time of 2500 ms (simple level) and 4000 ms (complex level) was set to remove any anticipatory or unreasonably slow response times. The test has previously been shown to be reliable for response times in both the simple and complex ($r > 0.85$) levels (Bandelow et al., 2011).
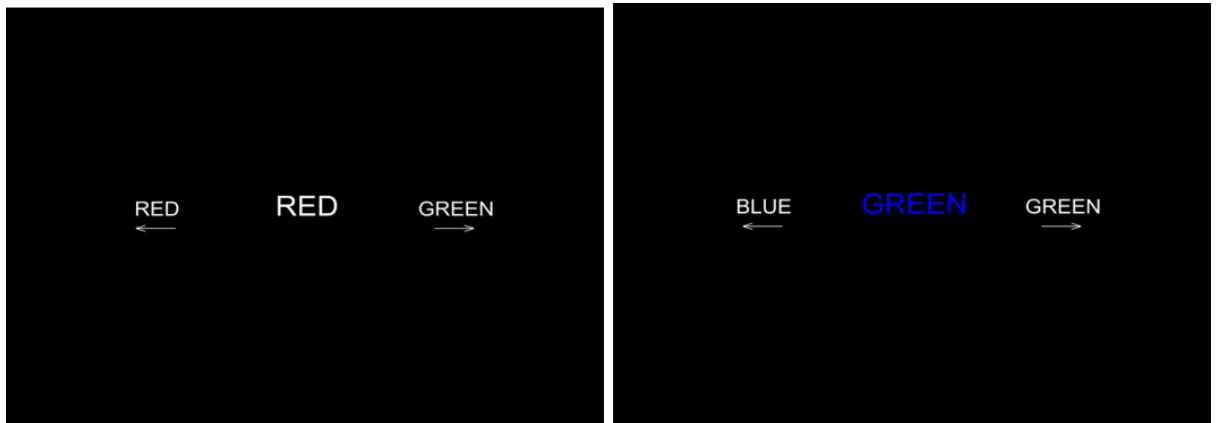
Figure 3.4. Example of simple (left) and complex (right) stimuli for the Stroop test.

3.6.7. Rapid Visual Information Processing (RVIP) Task

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

3.7. Standardised questionnaires

3.7.1. Mood questionnaires

It has been stated that mood may play a causal role in the changes in cognitive performance surrounding the menstrual cycle or with nutritional manipulations (Rogers, Edwards, Green, & Jas, 1992), therefore it is important that it is assessed when examining these constructs. Mood was assessed with paper-based questionnaires by the Profile of Mood States (POMS; McNair, Lorr, & Droppleman, 1971; Appendix 2) in Chapter 7 and the Brunel Mood Scale (BRUMS; Appendix 3) in Chapter 10, an altered short-form version of POMS (Terry, Lane, Lane, & Keohane, 1999). The short-form version was administered in Chapter 10 to reduce

the time demands on participants, due to the already onerous testing loads on these participants, and as the BRUMS is also a reliable measure of mood (Terry et al., 1999).

POMS is a standard validated psychological test where participants were presented with 65 words/statements that describe the feelings people have. For each word/statement, participants circled a number from 1-5 indicating whether they were currently experiencing that feeling "Not at all", "A little", "Moderately", "Quite a lot" or "Extremely". Each word/statement is associated with one of 6 categories; Tension, Depression, Anger, Fatigue, Confusion and Vigour, with some words not used for scoring purposes (Table 3.1). Results were added to provide a score for each category and Total Mood Disturbance (TMD) was calculated by adding the scores for Tension, Depression, Anger, Fatigue and Confusion and then subtracting the score for Vigour. POMS is appropriate for adults over the age of 18 years, without intellectual impartments and internal consistency of the POMS ranges from 0.84 to 0.95 (McNair et al., 1971).

Table 3.1. POMS categories and the associated words/statements.

Tension	Tense, Shaky, On Edge, Panicky, Relaxed, Uneasy, Restless, Nervous, Anxious
Depression	Unhappy, Sorry for things done, Sad, Blue, Hopeless, Unworthy, Discouraged, Lonely, Miserable, Gloomy, Desperate, Helpless, Worthless, Terrified, Guilty
Anger	Angry, Peeved, Grouchy, Spiteful, Annoyed, Resentful, Bitter, Ready to fight, Rebellious, Deceived, Furious, Bad tempered
Fatigue	Worn out, Listless, Fatigues, Exhausted, Sluggish, Weary, Bushed
Confusion	Confused, Unable to concentrate, Muddled, Bewildered, Efficient, Forgetful, Uncertain about things
Vigour	Lively, Active, Energetic, Cheerful, Alert, Full of pep, Carefree, Vigorous
Non-scored words	Friendly, Clear headed, Considerate, Sympathetic, Helpful, Good natured, Trusting

BRUMS is a short-form, validated version of POMS (Terry et al., 1999). In this version, participants were provided with 24 words/statements, corresponding to the same 6 categories as in POMS (Table 3.2). This short-form was demonstrated to be suitable for use in an adult population (Terry, Lane, & Fogarty, 2003).

Table 3.2. BRUMS categories and the associated words/statements.

Tension	Panicky, Nervous, Anxious, Unhappy
Depression	Unhappy, Depressed, Downhearted, Miserable
Anger	Angry, Annoyed, Bitter, Bad tempered
Fatigue	Worn out, Exhausted, Sleepy, Tired
Confusion	Confused, Muddled, Uncertain, Mixed-up
Vigour	Lively, Active, Energetic, Alert

3.7.2. Sleep questionnaire

The Pittsburgh Sleep Quality Index (PSQI; Appendix 4) is a paper-based questionnaire used to measure self-reported sleep quality that has been validated in both clinical populations and the general population (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). It consists of a total of 19 questions, generating scores in 7 components; subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. Each of these components is scored between 0 and 3, resulting in possible PSQI scores of 0-21. Overall PSQI scores > 5 have been considered to be indicative of poor sleep quality (Buysse et al., 1989). Sleep quality was measured due to the well-known effects of poor sleep quality on cognitive performance (Alhola & Polo-Kantola, 2007), in order to assess whether any changes in cognition across experimental studies could be ascribed to changes in sleep.

3.7.3. International Physical Activity Questionnaire (IPAQ)

The short-form version of the IPAQ is a validated self-report physical activity questionnaire (Craig et al., 2003) and was used to determine current physical activity level of participants (Appendix 5). Participants were asked to record the number of days that they performed vigorous physical activity, moderate physical activity, or walked for greater that 10 minutes in the previous week and the average duration of each of these activities. Metabolic equivalent (MET) scores of 3.3, 4.0 and 8.0 were applied to walking, moderate and vigorous physical activity respectively and each was multiplied by the number of days and average duration to produce a total METs value (Craig et al., 2003).

3.7.4. SCOFF eating disorder questionnaire

The SCOFF questionnaire consists of five questions used to indicate the likelihood of eating disorders (Figure 4) and was used in chapters to characterise the study population. Each

question can be answered with a YES or NO and a score of ≥ 2 YES responses indicates a likely case of anorexia nervosa or bulimia. Sensitivity for detecting anorexia nervosa and bulimia was 100% with a 12.5% false-positive rate in healthy controls (Morgan, Reid, & Lacey, 1999).

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1. Do you make yourself **S**ick because you feel uncomfortably full?
2. Do you worry that you have lost too much **C**ontrol over how much you eat?
3. Have you recently lost more than **O**ne stone (14lb) in a 3-month period?
4. Do you believe yourself to be **F**at when others say you are too thin?
5. Would you say that **F**ood dominates your life?

Figure 3.5. Questions included in the SCOFF eating disorder questionnaire.

3.8. Blood sample procedure and biochemical analysis

3.8.1. Sampling

Blood was drawn from an antecubital forearm vein and separated into ethylenediaminetetraacetic acid (EDTA) and serum tubes. EDTA tubes were immediately centrifuged at 3000 rev·min^{-1} at 4°C for 10 minutes and plasma was transferred into Eppendorf tubes and frozen at -80°C. Serum tubes were left to clot at room temperature for 30 minutes, before being centrifuged at 3000 rev·min^{-1} at 4°C for 10 minutes and serum was transferred into Eppendorf tubes and frozen at -80°C.

3.8.2. Biochemical analysis

Analysis of samples was conducted in the Norwich Medical School at the University of East Anglia. Oestradiol, PN1P, β-CTX and T_3 were analysed at the using an electro-chemiluminescence immunoassay (ECLIA; Roche, USA) and BAP was analysed using an enzyme-linked immunosorbent assay (ELISA; Microvue, USA) on a COBAS e501 analyser (Roche, USA). Inter-assay coefficient of variation (CV) for oestradiol was < 4.3% between 150-3000 pmol·L^{-1} with a detection limit of 18.4-1581 pmol·L^{-1}. Inter-assay CV for P1NP was < 3% between 20-600 µg·L^{-1} with a sensitivity of 8 µg·L^{-1}. Inter-assay CV for BAP was 5.8%, with a detection limit of 0.7 U·L^{-1}. Inter-assay CV for β-CTX was < 3% between 0.2 and 0.15 µg·L^{-1}, with a sensitivity of 0.01 µg·L^{-1}. Inter-assay CV for T_3 was < 1% between 2.0 and 3.1 nmol·L^{-1} with a detection limit of 0.3 nmol·L^{-1}.

Chapter 4.0. Reliability of muscle function measures

4.1. Introduction

In Chapter 6 and Chapter 9, custom-built dynamometers were used to measure MVIF of the quadriceps and FDI muscle, and CMJA was measured using a jump mat in Chapter 6. While the reliability of jump mats for CMJA has been described previously (Markovic et al., 2004), the reliability of the custom-built dynamometers used in this thesis has not been reported elsewhere so the current chapter will assess the reliability of these measures. Reliability data for the FDI muscle were published in the Journal of Sports Sciences (Martin, Cooper, Sale, Compton, & Elliott-Sale, 2015).

Commercial dynamometers are most commonly used to determine quadriceps, hamstring and elbow flexor isometric force (Agopyan et al., 2013; Farthing & Chilibeck, 2003; Lund et al., 2005; McKinnon, Graham, & Tiidus, 2012; de Carvalho Froufe Andrade et al., 2013; Silva et al., 2013) and have repeatedly been shown to be reliable measures of strength (ICC = 0.88-97, CV = 4.4-5.5%; Madsen, 1996; Maffiuletti, Bizzini, Desbrosses, Babault, & Munzinger, 2007; de Carvalho Froufe Andrade et al., 2013). The cost of these dynamometers can be prohibitive, so custom-built dynamometers are frequently used to measure muscle force.

Several authors (Blacker, Fallowfield, & Willems, 2013; Hamada, Sale, MacDougall, & Tarnopolsky, 2000; Kalmar & Cafarelli, 2006) have used purpose-built isometric rigs to assess quadriceps MVIF, although the reliability of these dynamometers is rarely reported. Blacker et al., (2013) demonstrated that with repeated MVIF measurements of the quadriceps, the limits of agreement (LoA) for their custom-built dynamometer was 12.7%. This is the only study to document reliability of a custom-built dynamometer for quadriceps MVIF measurements and presents wider confidence limits than other isometric force assessments (6.9 – 8.4% LoA; Widler et al., 2009). Further research is required to determine the appropriateness of using custom-built quadriceps dynamometers, and the reliability should be reported for each laboratory as this may vary dependent upon the design and set-up of each machine.

Custom-built dynamometers are also commonly used to measure MVIF of the FDI muscle (Allen & Doherty, 2011; Elliott et al., 2005a; Greeves et al., 1997; Kidgell & Pearce, 2010; Ranatunga, Sharpe, & Turnbull, 1987; van Duinen, Renken, Maurits, & Zijdewind, 2008; Zhou, Li, & Zev Rymer, 2013) as it is solely responsible for abduction of the index finger (Doyle & Botte, 2003) and can be fully recruited during voluntary contractions (Rutherford & Jones, 1988). Furthermore, the FDI muscle is rarely exposed to loading, so provides a relatively high level of control compared to other muscles that may be affected by habitual

loading. FDI muscle MVIF measurements have been shown to have good reliability in adults with peripheral nerve injuries (Schreuders, Roebroeck, Jaquet, Hovius, & Stam, 2004; ICC = 0.98) and children (Molenaar, Selles, Schreuders, Hovius, & Stam, 2008; ICC = 0.94 - 0.95), although the reliability in healthy adults is not yet clear.

The quadriceps and FDI muscle represent lower and upper body, large and small, and habitually loaded and unloaded muscles and, as such, provide useful, comprehensive and practical muscle force data. The aim of this study was to determine reliability of MVIF measurements for the quadriceps and FDI muscle, as these measures are used in Chapters 6 and 9.

4.2. Methods

4.2.1. Participants

Twenty-seven participants, 13 males (age 22 ± 6 years; height 1.80 ± 0.05 m; body mass 77.5 ± 6.7 kg) and 14 females (age 24 ± 5 years; height 1.65 ± 0.05 m; body mass 65.1 ± 9.4 kg) volunteered to take part in this study. All participants completed an informed consent form and health screen prior to participating and could withdraw from the study at any time without explanation. The study was approved by the Nottingham Trent University Research (Humans) Ethics Committee. Participants were excluded from the study if they had any muscular disorders or recent injuries that could influence muscle force production.

4.2.2. Experimental design

Quadriceps and FDI muscle MVIF were determined on two occasions (Trial 1 and Trial 2), following the same protocol on each visit, with the same experimenter collecting all data. There was a minimum of 1 day and a maximum of 7 days between testing sessions, which were conducted at the same time of day to prevent any effects of diurnal variation on muscle force production (Martin, Carpentier, Guissard, van Hoecke, & Duchateau, 1999).

Participants were asked to replicate their diet and activity patterns and abstain from strenuous exercise and alcohol consumption in the 24 h preceding each measurement, and refrain from consuming caffeine in the 4 h prior to each laboratory visit to prevent any effects on muscle force production (Ryan et al., 2013). Height (Section 3.3.1) and body mass (Section 3.3.1) were measured prior to Trial 1 only.

4.2.3. Experimental protocol

Quadriceps force (Section 3.4.2) and FDI muscle force (Section 3.4.3) were measured as previously described.

4.2.4. Statistical analysis

Data were checked for normality using Kolmogorov-Smirnov tests and Pearson's correlations were used to check data for proportional bias and homoscedacity (Atkinson & Nevill, 1998). Trial 1 and Trial 2 were compared using paired samples t-tests, CV, ICC (2-way mixed, absolute agreement) and systematic bias with ratio LoA (Bland & Altman, 1986). Effect sizes were calculated using Cohen's d (Cohen & Jacob, 1992) and were described as trivial (0.0 – 0.19), small (0.20 – 0.49), medium (0.50 – 0.79) and large (> 0.80). Data were analysed using SPSS (v21.0, IBM, USA) and Excel (Office 2016, Microsoft, USA). Data are presented as mean ± 1SD and the level of significance was set at $P \leq 0.05$.

4.3. Results

4.3.1. Quadriceps

Quadriceps MVIF was not different between Trial 1 and Trial 2 (499.9 ± 131.5 and 493.0 ± 130.7 N; P = 0.11, d = 0.26). Intra-class correlation between trials was r = 0.992 (0.980 to 0.996), with a CV of 3.21% and systematic bias and RLoA of 1.01 x/÷ 1.09 (Table 4.1). For a typical quadriceps force measurement of 496.5 N, there is a 95% likelihood that a second measurement would have a value between 453.7 and 539.3 N.

4.3.1. First Dorsal Interosseus muscle

There was no difference between Trial 1 and Trial 2 (31.8 ± 7.6 and 31.6 ± 7.3 N; P = 0.63, d = 0.03) for FDI muscle MVIF. Intra-class correlation between trials was r = 0.990 (0.978 to 0.995), with a CV of 3.22%. Ratio systematic bias and limits of agreement values were 1.00 x/÷ 1.09 (Table 4.1). For a typical FDI muscle force measurement of 31.7 N, there is a 95% likelihood that a second measurement have a value between 28.9 and 34.5 N.

Table 4.1. Reliability measures for quadriceps and first dorsal interosseus muscle measurements.

	Quadriceps force	FDI muscle force
Measure 1	499.9 ± 131.5 N	31.8 ± 7.6
Measure 2	493.0 ± 130.7 N	31.6 ± 7.3
Measure 1 (ln)	6.2 ± 0.3	3.4 ± 0.2
Measure 2 (ln)	6.2 ± 0.3	3.4 ± 0.2
Systematic bias	1.01	1.00
x / ÷ Ratio LoA	1.09	1.09
CV (%)	3.21	3.22
ICC (CI)	0.992 (0.908 to 0.996)	0.990 (0.978 to 0.995)
T-test	0.11	0.63
Variation LoA	496.5; 453.7, 539.3	31.7; 28.9, 34.5
Variation CV	496.5; 480.6, 512.5	31.7; 30.7, 32.7

Confidence interval; CI; Coefficient of variation, CV; First dorsal interosseus, FDI; Intraclass-correlation coefficient, ICC; Limits of agreement, LoA

4.4. Discussion

The methods used to measure quadriceps and FDI muscle MVIF were reliable and are suitable for use in future studies. Quadriceps force measurements were not significantly different between measures (P = 0.11). Ratio limits of agreements (9.0%) were similar, yet slightly lower than previous data (12.7%; Blacker et al., 2013) suggesting that this test is more able to detect differences in muscle strength. Quadriceps force data also had low CV (3.21%) and high ICC (0.992) values. These values compare favourably to other isometric quadriceps force measurements with commercially available dynamometers (ICC = 0.92 - 0.97, CV = 4.4 – 5.5%; (Maffiuletti et al., 2007; de Carvalho Froufe Andrade et al., 2013; Petersen, Hansen, Aagaard, & Madsen, 2007).

FDI muscle force measurements were not different between trials (P = 0.63), indicating no systematic bias and narrow agreement ratios (1.00 x/÷ 1.09). This is the first study to determine reliability of FDI muscle force measurements in healthy adults, in addition to demonstrating high ICC (0.990) and a low CV (3.22%) values. These results show better reliability than previous techniques assessing force in other intrinsic muscles in the hand (abductor pollicis ICC = 0.89; Liu, Carlson, & Watson, 2000) and arm (handgrip ICC = 0.476 – 0.954, P = 0.01; Clerke, Clerke, & Adams, 2005).

These data show that the measures used in the current study are reliable and suitable to use when assessing muscle force, and therefore will be used in Chapter 6 to assess the effects of reproductive hormones on muscle force, independent of changes in energy availability. Measurements of the quadriceps, which are habitually loaded during everyday activities and exercise, and the FDI muscle, which is rarely loaded, provide useful measurements to differentiate the site-specific, or whole-body effects of exercise-based interventions on muscle force production, which will be used in Chapter 9 to examine the effects of exercise-induced low energy availability.

Chapter 5.0. Period prevalence and perceived side effects of hormonal contraceptive use and the menstrual cycle in elite athletes

5.1. Introduction

During the menstrual cycle, there are fluctuations in reproductive hormone concentrations (Stricker et al., 2006) which may affect athletes' health and performance (Constantini, Dubnov, & Lebrun, 2005). With HC use, exogenous hormones are supplied to the system which down-regulates endogenous reproductive hormone concentrations and may have further implications for the health and performance of athletes (Martin, & Elliott-Sale, 2016). The Triad states the importance of reproductive hormones for bone health (De Souza et al., 2014) as the most severe negative bone metabolic outcomes are observed in those that are oestrogen and energy deficient (De Souza et al., 2008). It is not clear how the reproductive hormone environment affects the response to low energy availability for other health and performance factors outlined in the RED-S model (Mountjoy et al., 2014). As such, it is important to know the prevalence of different models of reproductive function in athletes, so that the corresponding reproductive profiles can be investigated in future studies. Data contained within this chapter has previously been published in the International Journal of Sports Physiology and Performance (Martin, Sale, Cooper, & Elliott-Sale, 2017).

Previous research has estimated that 40.2% (Torstveit & Sundgot-Borgen, 2005) and 46% (Brynhildsen et al., 1997) of elite female athletes use OCs, which was significantly greater than in the general population (27%; Torstveit & Sundgot-Borgen, 2005). This may be due to several factors, including HCs reducing symptoms of dysmenorrhea that may affect performance (Bruinvels et al., 2016; Chantler et al., 2009; Imai, Matsunami, Takagi, & Ichigo, 2014; Wong, Farquhar, Roberts, & Proctor, 2009) and the ability to manipulate menstruation around training and competition with OC use (Schaumberg et al., 2017). Previous research in elite athletes has only reported OC use and has not considered other delivery methods of HCs or detailed the preparations used by participants, which influence endogenous hormone concentrations and other physiological processes (Elliott-Sale et al., 2013; Godsland et al., 1990; Van Den Heuvel et al., 2005). Research in Italian athletes has reported the types of preparation used in combined OC users (n = 42), although these were mostly (85.4%) regional level athletes and therefore do not represent an elite athlete population. Furthermore, the reasons why athletes use HCs, discontinue HC use, and the perceived side effects of HC use have not been studied. The current lack of understanding of these perceived side effects is a barrier to implementing strategies to support/advise athletes and promote optimal health and performance. The aim of this study was to identify (1) the period prevalence of HC use, (2) the reasons for initiation and discontinuation of HCs and (3) the perceived side effects experienced by HC users and non-users in an elite athletic population. Characterising the HC use of female athletes will also identify the most common reproductive hormone models in

94

this population, which can be used to inform the populations investigated in subsequent studies (Chapters 6-10).

5.2. Method

5.2.1. Participants

A total of 476 female athletes were recruited to take part in the study through liaison with National Governing Bodies, coaching and support staff, or by direct contact with athletes, between January 2015 and December 2016. Athletes had to compete at a professional (full-time and salaried), international or national level and be aged over 18 years. Thirty-six athletes were excluded from the study for not meeting the criteria (Figure 5.1), resulting in a final sample of 430 athletes that competed at a professional/international (n = 361) or national (n = 69) level from 24 sports. Participants were supplied with a participant information sheet (Appendix 9) and all participants provided written informed consent (Appendix 10). The study was approved by the Nottingham Trent University non-invasive ethics committee.

5.2.2. Questionnaire

A questionnaire was designed specifically for this study (Appendix 11), which was only administered in paper-based form to improve the likelihood that all responses received were from the targeted population (Wright, 2006). Data provided by athletes were self-reported and reflect their experiences and perceptions. Participants were asked to provide demographic information (age, height, body mass and age of menarche), details regarding their competitive history (sport, level, duration competing at current level) and training details (frequency and duration; Table 5.1). Following this, participants were directed to complete different sections of the questionnaire depending on whether they currently used, or did not use, HCs. Non-HC users were asked to state whether they used an intra-uterine device (IUD), the typical duration and variability in length of their menstrual cycle, and whether they experienced symptoms or avoided exercise/training during their cycle. If applicable, participants were directed to state the reasons/symptoms and time points of the menstrual cycle when these occurred. Current HC-users were asked to detail the delivery method, preparation and duration of use for their current HC. Participants were asked whether they have discussed their current HC use with their coach/team doctor and whether the coach/team doctor was involved in the decision to use this HC. For their current HC, participants were asked to state the reasons for using this method, whether they considered any potential side effects before use, and whether they had experienced any negative or positive side effects. All participants were then asked to record any previous use of HCs, stating the delivery method, preparation, duration of use and reason for discontinuation for all HCs used.

95

5.2.3. Data analysis

Microsoft Excel (2016) and IBM SPSS (v. 23.0) were used to analyse data. Data were searched for non-unique dates of birth and, where duplicate values were observed, responses were checked to assess whether these were from different individuals. Athletes were categorised by competitive level (national or international/professional) to conduct a stratified analysis. For open-ended questions, a content analysis was conducted independently by two researchers (DM, KES) to categorise responses, whereby a frequency analysis was performed, which was checked for consistency. Differences between the researchers were resolved by discussion until a consensus was reached. Direct verbatim quotes were used to inform interpretation in some instances. Assumptions of normality were checked using the Shapiro-Wilk test and between group differences were examined using independent samples t-tests, Mann-Whitney U tests and Kruskal Wallis H tests. Effect sizes were calculated using Cohen's d (Cohen & Jacob, 1992) and were described as trivial (0.0 – 0.19), small (0.20 – 0.49), medium (0.50 – 0.79) and large (> 0.80). Pearson's chi-squared analyses were used to examine the relationships between categorical variables, with Fishers exact tests used where <80% of expected cell counts were > 5 (Quinn & Keogh, 2002). Data are represented as mean ± 1SD, frequencies and percentages and statistical significance was set at $P \leq 0.05$.

5.3. Results

Three hundred (69.8%) athletes reported using HCs at some point, with 49.5% of athletes currently using HCs and 50.5% not currently using any form of HC (Figure 5.1). Hormonal contraceptive users had a lower age of menarche (P = 0.010; d = 0.29) and length of time competing at current level (P = 0.048; d = 0.18) compared to non-HC users (participant characteristics in Table 5.1). Competitive level did not influence the prevalence of HC use (P > 0.05).

5.3.1. Menstrual cycle (non -hormonal contraceptive users)

Three athletes described themselves as amenorrheic, although the questionnaire did not specifically ask this question. Thirty-four athletes did not report their menstrual cycle length or did not provide enough information to interpret a response. Mean cycle length for the remaining athletes that reported having a menstrual cycle was 29 ± 5 d. Eight athletes reported a mean menstrual cycle duration of greater than 35 days and three athletes reported a mean menstrual cycle duration of less than 21 days. One-hundred and four (48.6%) athletes stated that their menstrual cycle was non-variable in length, while 110 (51.4%) athletes reported their cycle length to be variable with a mean variation of 9 ± 9 d. Copper IUDs were used by 2

participants (0.9%); with a mean menstrual cycle length of 28 ± 4 d. Menstrual cycle-related negative symptoms were reported by 168 athletes (77.4%) and categorical frequencies are presented in Table 5.2. Symptoms were experienced in the week prior to menstruation (25.0%), during days 1 and 2 of menstruation (81.6%) and between day 3 and the end of menstruation (28.9%). Nine athletes (4.1%) reported that they had to refrain from exercise at certain points of their menstrual cycle. Reasons included pain (n = 4), sickness (n = 2), or other reasons (n = 3), such as "Literally struggle to get out of bed so training is out of the question" or "at the beginning of the menstrual cycle I avoid to do tough session [sic]". Four athletes reported that they didn't refrain from exercise, although they provided additional comments stating "No – but only because I can't", "but struggle with contact [rugby]", "but I get back cramps 1 week before when running" and "I don't avoid it but I do sometimes have to delay things until cramps calm down". One athlete stated that "If anything I have to increase it [exercise]. Helps to pass quicker by maybe a day and helps the pain".

5.3.2. Hormonal contraceptive use

Combined HCs comprised 68.5% of HC use, with 30.0% using progestin-only and 1.9% using an unspecified type of OC. There was no difference in length of current HC use between combined (4.6 ± 3.7 years) and progestin-only HC users (3.9 ± 4.4 years; P = 0.193; d = 0.17), or between different delivery methods (P = 0.649). Oral contraceptives were the most widely used (78.4%), followed by the implant (13.1%), injection (3.8%), IUS (2.8%) and vaginal ring (0.5%), with one participant using a combination of the implant and OC. All combined OCs were monophasic and contained EO as the oestrogenic component in varying doses: 20 μg (n = 4, 2.8%), 30 μg (n = 116, 80.0%), 35 μg (n = 19, 13.1%). Six participants (n = 4.1%) used combined preparations but did not specify the oestrogenic dose. Twelve different progestins were used in various doses, with LNG accounting for 51.4% of progestin use.

The most common reason athletes chose their specific type/delivery method was ease of use (18.8%), and the most common side effects considered prior to HC use were weight gain (33.0%) and mood changes/swings (12.7%). The side effects experienced by HC-users are shown in Table 5.4. Negative side effects were significantly more common with progestin-only HCs (39.1%) compared to combined HCs (17.8%; P = 0.001) and were significantly more common in the implant (53.6%) compared to other delivery methods (P = 0.004; Table 5.5). Type and delivery method of HC did not affect the prevalence of reported positive effects (P > 0.05). HC users were significantly more likely to report positive effects of HCs than negative effects (P < 0.05).

International/professional athletes were significantly more likely to discuss HC use with their coach/team doctor (25%) compared to national level athletes (0%; P < 0.001). Competitive level did not influence coach/team doctor involvement in the decision to initiate HC use (P = 0.070), although this did occur for 7.6% of international/professional athletes and no national level athletes. The coach/team doctor was involved in the decision to use HCs for 14 (6.6%) athletes, of which 12 used OCs and 2 used an implant. Ultra-low dose EO (20 µg) OCs accounted for 25% of OC use in this group, in comparison to 2.7% of overall OC use, which was a significant effect (P = 0.010). Where the coach/team doctor was involved in the decision, athletes stated that they were prescribed these HCs for contrasting reasons including; 'Higher level of oestrogen", "Apparently lowest oestrogen", "Low hormones" and "In attempt to reduce monthly fluctuations in my performance and fatigue".

In total, 87 (40.1%) non-HC users had previously used some form of HC, with 64 (30.0%) current HC users previously using a different HC. There were 218 incidences of previous HC use, as some athletes had used 2 (n = 49), 3 (n = 13), 4 (n = 4) and 5 (n = 1) previous types of HC. Combined OCs accounted for 78.4% of previous use, with progestin-only OCs (7.8%), implant (7.8%), injection (6.0%) and IUS (1.8%) also used. The reasons provided for discontinuation of previous HCs are presented in Table 5.6. Mean duration of previous HC use was 2.2 ± 2.3 years, with no difference between types (P = 0.360; d = 0.17) or delivery methods (P = 0.733).

Excluded
No consent (n = 2)
<18 y (n = 3)
Duplicate completion (n = 5)
Insufficient completion (n = 6)
Non-elite athlete (n = 26)
Non-UK based (n = 4)

Total number of questionnaire responses (n = 476)

Responses included in analysis (n = 430)

Hockey	n = 103	Judo	n = 11	Triathlon	n = 4
Football	n = 83	Kayak	n = 8	Short track	n = 4
Rugby	n = 55	Basketball	n = 8	Table tennis	n = 3
Athletics	n = 32	Boxing	n = 7	Taekwondo	n = 3
Rowing	n = 25	Badminton	n = 7	Trampoline	n = 1
Cricket	n = 22	Canoe	n = 6	Swimming	n = 1
Netball	n = 21	Diving	n = 6	Cycling	n = 1
Squash	n = 12	Archery	n = 6	Orienteering	n = 1

Copper IUD (n = 2)

Currently non-HC users (n = 217)

Current HC users (n = 213)

Never used HCs (n = 130)

Previously used HCs (n = 87)

Progestin only HCs (n = 64)

Unspecified OC brand (n = 4)

Combined HCs (n = 146)

OC
(n = 22)
Cerazette® (n=10)
Cerelle® (n=8)
Noriday® (n=3)
Nacrez® (n=1)

Implant
(n = 28)
Nexplanon® (n=11)
Norplant® (n=10)
Not specified (n=5)
Implanon® (n=2)

Injection
(n = 8)
Depo Provera® (n=8)

IUS
(n = 6)
Mirena® (n=6)

Vaginal ring
(n = 1)
NuvaRing® (n=1)

OC
(n = 145)
Microgynon® (n=52) Femodene® (n=2)
Rigevidon® (n=27) Dianette® (n=2)
Cilest® (n=16) Milinette® DNS (n=2)
Yasmin® (n=9) Anastrella® (n=1)
Levest® (n=8) Gedarel® 20 (n=1)
Marvelon® (n=8) Gedarel® 30 (n=1)
Ovranette® (n=6) Ogestrel® (n=1)
Loestrin® 20 (n=4) Loestrin® DNS (n=1)
Gedarel® DNS (n=3) Demulen® (n=1)

Figure 5.1. The prevalence of type, delivery method and preparation of hormonal contraceptives (HCs) used and the prevalence of non-HC use. Dose not specified, DNS; Intrauterine device, IUD; Intrauterine system, IUS; Oral contraceptive, OC.

Table 5.1. Participant characteristics for hormonal contraceptive (HC) users and non-HC users. * Indicates a significant difference between HC users and non-HC users (P < 0.05).

Demographic information	HC users	Non HC users	Total
Age (y)	24.1 ± 4.5	24.3 ± 4.3	24.2 ± 4.4
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
Weight (kg)	66.2 ± 9.8	66.0 ± 9.3	66.1 ± 9.6
Body mass index (kg·m^2)	23.1 ± 2.6	23.0 ± 2.5	23.1 ± 2.5
Age at menarche (y)	13.4 ± 1.5	13.8 ± 1.3	13.6 ± 1.4*
Gynaecological age (y)	10.7 ± 4.6	10.6 ± 4.6	10.6 ± 4.6
Duration competing at current level (y)	5.0 ± 3.6	5.7 ± 4.1	5.4 ± 3.9*
No. training sessions per week	8.5 ± 4.5	8.4 ± 4.0	8.5 ± 4.3
Mean training session duration (mins)	92.8 ± 29.8	89.1 ± 27.8	90.9 ± 28.8
Total weekly training duration (mins)	769.7 ± 440.8	720.3 ± 385.6	744.6 ± 413.9

Table 5.2. Frequency and prevalence of physical and emotional symptoms reported during the menstrual cycle for hormonal contraceptive users.

	Symptom	Frequency	Prevalence (%)
Physical	Stomach cramps/abdominal pain	103	47.5
	Unspecified cramp	48	22.1
	Back pain	37	17.1
	Headache/migraine	21	9.7
	Bloating	12	5.5
	Nausea/sickness/vomiting	10	4.6
	Tiredness/fatigue/lethargy	9	4.1
	Dizzy/lightheaded/lack of coordination	5	2.3
	Leg discomfort	4	1.8
	Unspecified pain	3	1.4
	Hot flushes/sweating	2	0.9
	Hunger/increased appetite	2	0.9
	Sore breasts	2	0.9
	Bad skin	1	0.5
	Constipation	1	0.5
	Heavy bleeding	1	0.5
	Muscle ache	1	0.5
	Problems with exercise	1	0.5
	Sore throat	1	0.5
	Tight neck	1	0.5
	Weakness	1	0.5
Emotional	Mood changes/swings	9	4.1
	Irritability	1	0.5
	Flustered	1	0.5

Table 5.3. Prevalence of side effects considered when commencing current hormonal contraceptive use.

	Side effects	Frequency	Prevalence (%)
Physical	Weight gain	71	33.3
	Altered cycle length	15	7.0
	Altered skin	15	7.0
	Headaches/migraine	11	5.2
	Altered bleeding	4	1.8
	Blood clots	4	1.8
	Nausea/sickness/vomiting	4	1.8
	Tiredness/fatigue/lethargy	4	1.8
	Altered period pain	3	1.3
	Effect on training/performance	3	1.3
	Increased cancer risk	3	1.3
	Water retention	3	1.3
	Altered appetite	2	0.9
	Breast issues	2	0.9
	Hormone imbalance	2	0.9
	Procedural issues	2	0.9
	Unspecified pain	2	0.9
	Bloating	1	0.5
	Bone density	1	0.5
	Cramps	1	0.5
	Dizziness	1	0.5
	Hair loss	1	0.5
	Low sex drive	1	0.5
	Stroke	1	0.5
Emotional	Mood changes/swings	27	12.7
	Depression	2	0.9

Table 5.4. Prevalence of reported negative and positive side effects for current hormonal contraceptive use.

	Negative effect	Frequency	Prevalence (%)	Positive effect	Frequency	Prevalence (%)
Physical	Weight gain	16	7.5	Regular period	27	12.7
	Irregular periods	9	4.2	Cessation of/less frequent periods	26	12.2
	Poor skin	6	2.8	Reduced bleeding/lighter periods	23	10.8
	Headaches/migraines	4	1.9	Improved skin	13	6.1
	Altered cycle length	3	1.4	Reduced period pain	10	4.7
	Breast issues (bigger/sore)	3	1.4	Reduced cramps (unspecified)	7	3.3
	Constant/irregular bleeding	3	1.4	Reduced pain (unspecified)	6	2.8
	Spotting	3	1.4	Reduced headaches/migraine	3	1.4
	Tiredness/fatigue/lethargy	3	1.4	Increased iron	3	1.4
	Effect on training/performance	2	0.9	Less ill/sick	3	1.4
	Nausea/sickness/vomiting	2	0.9	Resumption of cycle from amenorrhea	3	1.4
	Water retention	2	0.9	Reduced stomach cramps	3	1.4
	Abnormal liver function	1	0.5	Effect on training/performance	2	0.9
	Bloating	1	0.5	Reduced bloating	1	0.5
	Hormone imbalance	1	0.5	Improved bone density	1	0.5
	Increased appetite	1	0.5	Less faint	1	0.5
	Stomach pain	1	0.5	Reduced fluctuations in water retention	1	0.5
	Unspecified pain	1	0.5	Reduced fluctuations in weight	1	0.5
				Reduced PCOS side effects	1	0.5
Emotional	Mood changes/swings	9	4.2	Improved mood	3	1.4
Both				Helps PMT	1	0.5
Practical				Ability to predict/change cycle date	45	21.1
				Couldn't forget to take	3	1.4

Polycystic ovarian syndrome, PCOS; Pre-menstrual tension, PMT.

Table 5.5. Prevalence of reported negative and positive effects of hormonal contraceptive use in current users, separated by type and delivery method of hormonal contraceptive

	Type of hormonal contraceptive			Delivery method of hormonal contraceptive					
	Combined	Progestin-only	Total	OC	Implant	Injection	IUS	Vaginal ring	Total
Received Negative symptoms	26	25	51	35	15	2	2	0	54
Didn't receive negative symptoms	120	39	159	136	13	6	4	1	214
Percentage with symptoms (%)	17.8	39.1	24.4*	20.5	53.6	25.0	33.3	0.0	25.2*
Received positive effects	99	42	141	117	18	3	5	1	144
Didn't receive positive effects	47	22	69	54	10	5	1	0	70
Percentage with symptoms (%)	67.8	65.3	67.1	68.4	64.3	37.5	83.3	100.0	67.3

* Indicates a significant effect of type or delivery method ($P < 0.05$). Oral contraceptive, OC; Intrauterine system, IUS.

Table 5.6. Reasons, frequency and prevalence for discontinuation of previous hormonal contraceptives.

	Reason	Frequency	Prevalence (%)
Physical symptoms	Weight gain	28	12.8
	Headaches/migraine	18	8.3
	More frequent or heavier bleeding	13	6.0
	Irregular/no bleeding	10	4.6
	Poor skin	7	3.2
	Constant bleeding	6	2.8
	Fatigue/tiredness/lethargy	6	2.8
	Bone health	5	2.3
	Impaired training/performance/recovery	5	2.3
	Nausea/vomiting	4	1.8
	Resumption/regulation of menses	4	1.8
	Stomach cramps	3	1.4
	Stroke and cancer risk	3	1.4
	Water retention	3	1.4
	Cramps	2	0.9
	Hormone imbalance	2	0.9
	Impaired sleep	2	0.9
	Low libido	2	0.9
	Painful periods	2	0.9
	Bloating	1	0.5
	Blood pressure	1	0.5
	Blood side effects [sic]	1	0.5
	Breast pain	1	0.5
	Dizziness and blurred vision	1	0.5
	For oestrogen reasons [sic]	1	0.5
	Hot flushes	1	0.5
	Illness	1	0.5
	Pain during intercourse	1	0.5
	PMS	1	0.5
	Removed to assess oestrogen level	1	0.5
Emotional symptoms	Mood	29	13.3
	Wanting to be "normal" / "natural"	5	2.3
	Depression	4	1.8
	Needed a rest/break	3	1.4
Practical	Not sexually active/not needed	30	13.8
	Forgetting to take pill	16	7.3
	Doctor/nurse recommendation	11	5.0
	Didn't like it	10	4.6
	Pregnancy	6	2.8
	New preparation/type	4	1.8
	Ran out	4	1.8
	Went abroad/travelling	4	1.8
	Ineffective	3	1.4
	Wanted something different/permanent	2	0.9
	Word of mouth	2	0.9
	Loss of effect[sic]	1	0.5

5.4. Discussion

This study has shown that there is an approximately even prevalence of HC use and non-HC use in elite female athletes and the majority (69.8%) of female athletes have used HCs at some point in their sporting career. These results highlight the importance of understanding the effects of the menstrual cycle and HC use in elite sportswomen, especially for the most prevalent type (combined OC) and preparation (Microgynon®) of HC. This research also shows that, where the reproductive hormone environment may affect the physiological response, such as the response to low energy availability (De Souza et al., 2008), it is important compare the responses between HC users and non-users to appropriately represent the female athlete population.

The prevalence of HC use (49.5%) was higher than recent data for the general population of reproductive age in the UK (30.0%; Cea-Soriano et al., 2014) and USA (27.6%; Daniels, Daugherty, & Jones, 2014). In the general population, combined OCs accounted for 54.0% of HC use (Cea-Soriano et al., 2014), while in the current study it was 69.0%. Nearly a third of combined OC users perceived the ability to predict or manipulate menstruation, thereby avoiding menstruation during training or competition, as a positive effect, which may explain the differences in OC use between athletes and the general population. This is supported by research (Schaumberg et al., 2017) showing that 43.5% of competitive athletes manipulated their menstruation around training and competition frequently, which was greater than sub-elite (22.5%) and recreationally active (15.8%) women. Progestin-only HCs were used by 30% of athletes, with the implant (13.1%) and progestin-only OCs (10.3%) being the most widely used. The most common perceived positive effect of progestin-only HC use was a cessation of, or less frequent, bleeding, which was reported by almost 40.0% of athletes. While previous research has stated the prevalence of OC use in elite athletes (Brynhildsen et al., 1997; Torstveit & Sundgot-Borgen, 2005) and combined OC preparations in a predominately regional level athlete group (Cauci et al., 2016), this was the first study to document the prevalence of all types and delivery methods of HCs, in addition to the preparation of HCs in an elite athlete group, which enables the quantification of steroid hormone content and concentrations. Twelve different progestins were used in varying concentrations, while EO was the oestrogenic component used in all HCs. Microgynon® was the most prevalent preparation (n = 52), followed by Rigevidon® (n = 27), which contain similar oestrogen and progestin doses (both 30 µg EO and 150 µg LNG). Levonorgestrel was the progestin used in 64.8% of combined OCs, with Norgestimate (11.0%), and Desogestrel (9.0%) the other most frequently used. This stands in contrast to Italian athletes, where Gestodene (45.2%) and Drospirenone (21.4%) were the most prevalent progestin in 42 combined OC users, with only

7.1% containing LNG, emphasising the need to consider the region-specific prescription of hormonal contraceptives.

Four HC users were prescribed ultra-low dose (20 µg EO) OCs; with three cases involving the coach/team doctor in the decision to use this preparation, all of which were from different sports. Ultra-low dose OCs are associated with reduced headaches, nausea and breast tenderness compared to higher dose EO formulations (Vitzthum & Ringheim, 2005) and can reduce the symptoms of dysmenorrhea (Harada & Momoeda, 2016), so may have been prescribed to reduce these symptoms whilst maintaining the benefits of improved cycle control. These data reflect the HC use of UK-based athletes, which may be different to other countries where the use of other formulations such as extended cycle OCs is more prevalent (Hall & Trussell, 2012), therefore research should document the prevalence of HC use in other countries.

Combined HC users reported a lower number of perceived negative side effects (17.8%) compared to progestin-only HC users (39.1%), with the implant having a significantly higher incidence of reported negative symptoms compared to other delivery methods (Table 5.6). The most common consideration (33.3%) prior to initiating HC use was weight-gain, although only 7.5% reported increased weight gain which is lower than in the general population with HC use (34%; Nault, Peipert, Zhao, Madden, & Secura, 2013). Nineteen negative and 23 positive categories of side effects were identified, emphasising the individuality of responses and that athletes should be considered on a case by case basis. The most prevalent, positive side effects reported were the ability to predict/change menstruation (n = 45), having regular periods (n = 27) and cessation of/less frequent bleeding (n = 26), showing that changes to the timing, frequency and amount of bleeding with HC use were well-received. It should be noted that athletes were asked to state the non-contraceptive benefits of HC use, therefore the primary benefit and reason of HC use may have been for contraception.

Sixty-four (30.0%) HC users previously used a different form of HC and 87 (40.1%) non-HC users had previously used a form of HC. The most common reasons provided for discontinuation of HCs were: they were no longer needed (19.9%), they altered mood (19.2%), resulted in weight gain (18.5%) and caused headaches/migraines (11.9%). It is important to note that 46 separate reasons were provided for discontinuation of HCs, emphasising the high inter-individual response. This further emphasises that sport practitioners should openly discuss HC use and side effects with athletes to monitor athletes' health, well-being and performance.

107

Although exercise may reduce the occurrence and severity of dysmenorrhea (Daley, 2008b), 77.4% of non-HC users reported negative side effects associated with the menstrual cycle, which is similar to the general population (Ju et al., 2014). The most commonly reported side effects were stomach cramps (47.5%), unspecified cramps (22.1%), back pain (17.1%) and headaches/migraines (9.7%). Despite having physically demanding lifestyles, only 4.2% of athletes stated that they refrained from exercise at certain points of their menstrual cycle, which is lower than the general population where dysmenorrhea limits daily activities in 15-29% of women (Ju et al., 2014). This highlights the need to understand how changes in reproductive hormone concentrations across the menstrual cycle affect athletic performance. A recent study in HC users and non-users, showed 51.1% of athletes thought their menstrual cycle affected training and performance (Bruinvels et al., 2016), although the current data indicates that this rarely translates into athletes modifying training schedules to accommodate symptoms.

Twenty-four distinct, negative symptoms were reported by non-HC users (Table 5.2) and approximately half of the athletes reported menstrual cycle length variability with a relatively high mean variation of (9 ± 9 d) in these athletes. Although the current questionnaire did not ask specifically about amenorrhea, three athletes described themselves as amenorrheic, and it is recommended that future studies explicitly ask this question in order to not under-represent the occurrence of amenorrhea in elite sport. Side effects were mostly experienced during the first two days of menstruation (81.6%), however also occurred in the week prior to menstruation (25.0%) and between day 3 and the end of menstruation (28.9%). These data emphasise the individuality of responses and the importance of athletes monitoring their menstrual cycle and associated symptoms. These data show that athletes and coaches/support staff should maintain an open dialogue about the menstrual cycle and encourage flexibility in training schedules, when possible, to accommodate the most severe side effects.

This study has shown that, in the elite female athlete population, there is an approximately even prevalence of HC users and non-users, emphasising the importance of understanding the effects of both of these reproductive hormone models on physiological function. In particular, combined OC use (specifically Microgynon®) is the most common HC used and thus the most relevant HC to study in this population. Therefore, Chapters 6-8 will explore the effects of the menstrual cycle and Microgynon®, representing combined OC use, on muscle function, cognitive function and bone metabolism. These factors are suggested to be affected by low energy availability in the RED-S model (Mountjoy et al., 2014), although it is currently not

clear whether reproductive hormones affect these factors independent of energy availability. It is also unknown whether the reproductive hormone environment affects the response to low energy availability for muscle function and cognitive function, so this will be explored in Chapters 9-10 using eumenorrheic women and combined OC users, reflecting the reproductive hormone environment of the majority of female athletes.

109

Chapter 6.0. The effects of menstrual cycle phase and oral contraceptive use on muscle force production

6.1. Introduction

The RED-S model suggests that muscle strength may be impaired by low energy availability (Mountjoy et al., 2014), however it is not clear whether alterations to the reproductive axis, independent of low energy availability, will influence muscle force production (De Souza, Williams, et al., 2014). Chapter 5 identified that the menstrual cycle and combined OC use, specifically Microgynon®, were the most prevalent models of reproductive functioning in athletes, and these can be used to examine the effects of reproductive hormones on muscle force production, when in an energy replete state.

Numerous studies have shown that muscle force production and dynamic muscle performance do not change across the menstrual cycle (Ekenros, Hirschberg, Heijne, & Fridén, 2013; Elliott, Cable, Reilly, & Diver, 2003; Fridén, Hirschberg, & Saartok, 2003; Giacomoni, Bernard, Gavarry, Altare, & Falgairette, 2000a; Gür, 1997; Janse de Jonge, Boot, Thom, Ruell, & Thompson, 2001; Lebrun, McKenzie, Prior, & Taunton, 1995; Rechichi & Dawson, 2009). In contrast to these results, studies have shown that maximum force production occurs in the early- (Davies, Elford, & Jamieson, 1991a), mid- (Wirth & Lohman, 1982) and late-follicular phases (Phillips et al., 1996; Sarwar et al., 1996), the post-ovulatory phase (Bambaeichi et al., 2004) and the luteal phase (Ekenros et al., 2013). With some exceptions, (Davies et al., 1991a; Wirth & Lohman, 1982), significant changes in force production across the menstrual cycle are typically associated with elevated oestrogen concentrations (Bambaeichi et al., 2004; Phillips et al., 1996; Sarwar et al., 1996). Muscle force production has also been shown to be impaired in the mid-luteal phase only, when progesterone concentrations are elevated and oestrogen concentrations are relatively high (Tenan et al., 2016). Within the studies that show an effect of menstrual cycle phase on muscle force production, these effects occur at different time points of the menstrual cycle.

Differences in how menstrual cycle phases have been defined between studies and a poor level of methodological control make it difficult to compare the results of previous research. Many studies have not used gold-standard methods, such as taking blood samples to determine hormone concentrations or confirming ovulation using assays. Furthermore, dietary and exercise practices have not been well-controlled in the lead-in period prior to test sessions, which will influence muscle force production (Behrens et al., 2015; MacIntyre, Reid, Lyster, Szasz, & McKenzie, 1996; McLellan, Lovell, & Gass, 2011).

Previous research exploring the effects of OC use on muscle force production and dynamic performance have compared pill consumption days to the PFI (Elliott et al., 2005) or have

studied several different phases of an OC cycle (Ekenros et al., 2013; Nicolay et al., 2008; Rechichi & Dawson, 2009; Sarwar et al., 1996); reporting unchanged isometric muscle force production between different OC phases. In a measure of dynamic muscle performance, Rechichi and Dawson (2009) showed that reactive strength from a drop jump was significantly lower during the PFI, at a time when endogenous oestradiol concentrations were highest. These studies used non-homogenous groups, with participants using a variety of OC preparations (brands), which influences endogenous hormone concentrations and may lead to type II errors (Elliott-Sale et al., 2013). Therefore, any effects of OC use on isometric muscle force and dynamic muscle performance should be further evaluated in participants using the same OC preparation, which provides the same type and dose of synthetic oestrogen and progestin.

There are conflicting data regarding the effects of the menstrual cycle and OC use on muscle force production and dynamic muscle performance. Therefore, the aim of this study was to assess vertical jump height and isometric muscle force production of the Quadriceps and FDI muscle, which are reliable measures of dynamic and isometric force (Chapter 4; Markovic, Dizdar, Jukic, & Cardinale, 2004), at different phases of the menstrual cycle and OC cycle. This study aimed to address previous methodological flaws by confirming menstrual cycle phase using LH test kits, controlling pre-test diet and exercise, and by using a homogenous OC group (Microgynon®).

6.2. Method

6.2.1. Participants

Thirty-seven recreationally active participants were recruited to take part in the study (eumenorrheic, n=21; OC users, n=16). Seven eumenorrheic participants were unable to complete the study due to anovulatory cycles (n=4), menstrual cycle length > 35 days (n=1), relocation (n=1) and personal issues (n=1). Two OC users were unable to complete due to cessation of OC use (n=1) and blood sampling issues (n=1). These withdrawals resulted in a total of 14 eumenorrheic and 14 OC participants (Table 6.1). Questionnaires were used to assess menstrual cycle characteristics (Appendix 12) and OC use details (Appendix 13) in eumenorrheic and OC-using participants. Eumenorrheic participants had a regular menstrual cycle for at least 6 months prior to recruitment, with mean menstrual cycle duration of 28 ± 2 days. A low dose, combined OC preparation, with a regimen of 21 pill consumption days and a 7-day PFI (Microgynon®) was consumed by all OC users for a minimum of 6 months prior to recruitment to limit the occurrence of improper cycle regulation (Foidart et al., 2000). This preparation was identified as the most commonly used in elite athletes in Chapter 5. A

homogenous OC group using the same preparation was employed to reduced inter-participant variability (Elliott-Sale et al., 2013). The study was approved by the Nottingham Trent University Research (Humans) Ethics Committee (Reference number 280). Participants were provided with a participant information sheet (Appendix 14), completed a health screen (Appendix 8) and gave their written informed consent (Appendix 15) prior to commencing the study. Participants could withdraw from the study at any time.

6.2.2. Experimental design

Participants attended the laboratory for a familiarisation session, followed by three experimental trials, all following the same protocol. Eumenorrheic participants were tested during the early follicular phase (EF; day 2-3), ovulatory phase (OV; day immediately following a surge in luteinising hormone as confirmed by ovulation detection kit [Clearblue®]) and mid luteal phase (ML; 7-8 days following LH surge). These phases were used to represent three distinct profiles of oestradiol with low (EF), medium (OV) and high (ML) endogenous oestradiol concentrations. Oral contraceptive users were tested in the first week of pill consumption (pill consumption day 2-3; PC1), after two weeks of pill consumption (day 15-16; PC2) and during the PFI (day 3-4 PFI). Early (PC1) and late (PC2) pill consumption phases were used as circulating exogenous steroid hormone concentrations increase across pill-taking days (Carol et al., 1992; Kuhnz et al., 1992b, 1991). The PFI was used to represent a time when no exogenous hormones were supplied. The order of testing for both groups was determined by the participant's cycle (*e.g.,* the first testing session corresponded with the next testing time point following recruitment) and availability for testing (*e.g.,* a testing time-point could be completed the following cycle if the participant was unavailable) and as such did not follow a consistent order. Participants arrived at the laboratory at 08.00 (± 30 minutes), at the same time for each participant, having fasted from 22.00 the previous night and having consumed 600ml of water upon awakening. Oral contraceptive users were asked to consume their pill 1 h prior to arriving at the laboratory and were asked to consume it at this time for the duration of the study. Dietary intake and physical activity were recorded in the 24 h prior to the initial laboratory visit and participants were asked to replicate this in the day preceding each test session, which was verbally confirmed by the experimenter. Participants were asked to arrive at the laboratory in a rested state, having abstained from alcohol for a minimum of 24 h and caffeine for a minimum of 4 h.

6.2.3. Experimental protocol

6.2.3.1. Familiarisation and baseline measures

Participants underwent a familiarisation of the experimental protocol, followed by a body composition assessment. Sum of eight skinfolds (triceps, biceps, subscapular, iliac crest, supraspinale, abdominal, medial calf and front thigh), body mass index (BMI) and waist:hip ratio were measured to determine body composition using the ISAK restricted profile, by a level 1 qualified ISAK practitioner (Marfell-Jones et al., 2011; Section 3.2.2).

6.2.3.2. Main experimental protocol

Upon arrival to the laboratory, body mass (Section 3.3.1) was measured, before 30 ml of blood was drawn from an antecubital vein for measurement of oestradiol (Section 3.8.1). Participants then remained sedentary for ~90 minutes, during which time they were provided with a standardised breakfast containing $1g \cdot kgBM^{-1}$ carbohydrate, $0.1g \cdot kgBM^{-1}$ fat and $0.19g \cdot kgBM^{-1}$ protein and participants completed questionnaires and a cognitive test battery which will be discussed in Chapter 7. Quadriceps MVIF (Section 3.4.2), FDI muscle MVIF (Section 3.4.3) and CMJA (Section 3.4.4) measurements were then carried out in the same order for all experimental trials. Blood samples were analysed for oestradiol as described in Section 3.8.2.

6.2.4. Statistical analysis

An *a priori* power analysis indicated that 10 participants in each group were required for a study power of 80% ($1 – \beta = 0.8$) and 14 participants in each group were required for a study power of 95% ($1- \beta = 0.95$), at an alpha level of $P < 0.05$, based upon FDI force production values from previous research (Elliott et al., 2003). All data were checked for normality using the Shapiro-Wilk test. Demographic information were compared between groups using independent samples t-tests. Muscle force production and oestradiol were analysed independently for eumenorrheic and OC participants, using one-way repeated measures analysis of variance (ANOVA; Statistica v 13.0), with significant effects explored using Bonferroni adjusted t-tests. Where sphericity of data were violated, Greenhouse-Geisser adjustments were used. Effect sizes were calculated using Cohen's d (Cohen & Jacob, 1992) and were described as trivial (0.0 – 0.19), small (0.20 – 0.49), medium (0.50 – 0.79) and large (> 0.80). Pearson's correlation coefficients were used to examine relationships between oestradiol and muscle force production for eumenorrheic participants and OC users independently. Percentage differences in muscle force production between menstrual cycle phases and OC phases were calculated. Data are presented as mean ± 1SD and the level of significance was set at $P \le 0.05$.

6.3. Results

Age, height, body mass, BMI and Waist:hip were not different between eumenorrheic and OC participants (all P < 0.05), while sum of 8 skinfolds was significantly (P = 0.039; d = 0.83) higher in eumenorrheic (150.1 ± 42.6 mm) compared to OC users (120.2 ± 29.2 mm; Table 6.1).

Table 6.1. Demographic information for eumenorrheic participants and oral contraceptive users.

	Eumenorrheic n = 14	Oral contraceptive n = 14
Age (y)	21 ± 2	22 ± 4
Height (m)	1.65 ± 0.07	1.66 ± 0.06
Body mass (kg)	64.8 ± 10.1	61.1 ± 6.7
Body mass index (kg·m^2)	23.8 ± 3.5	22.1 ± 1.6
Waist:hip	0.71 ± 0.02	0.71 ± 0.05
Sum of 8 skinfolds (mm)	150.1 ± 42.6	120.2 ± 29.2*

* Indicates a significant difference between groups (P < 0.05)

For eumenorrheic participants, oestradiol concentrations were significantly different between menstrual cycle phases (main effect time; P < 0.05; Figure 6.1). EF phase oestradiol concentrations were significantly lower than OV (P = 0.02; d = 1.18) and ML phases (P < 0.001; d = 1.97) and ML phase oestradiol concentrations were significantly higher than the OV phase (P = 0.03; d = 0.76). For OC users, there was no significant effect of OC phase on oestradiol concentrations (P = 0.076), but there was a medium effect size when comparing PC1 to PC2 (d = 0.69, P = 0.25) and a large effect size when comparing PC2 to the PFI (d = 1.01, P = 0.075).
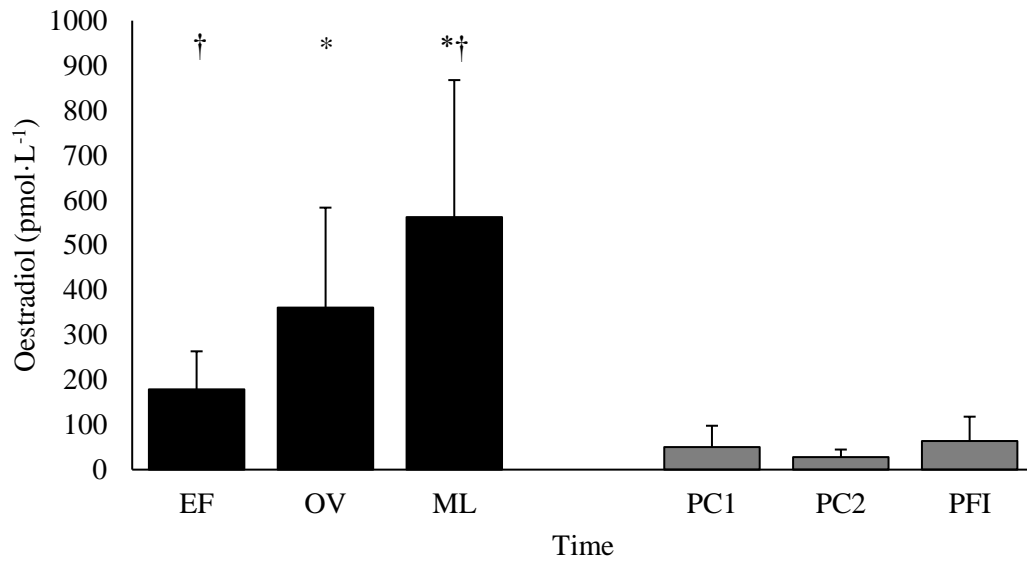
Figure 6.1. Mean ± 1SD oestradiol concentrations in eumenorrheic participants (black bars) in the early follicular (EF), ovulatory (OV) and mid-luteal (ML) phases and oral contraceptive users (grey bars) at first (PC1) and second (PC2) pill consumption time points and during the pill-free interval (PFI). * Indicates a significant difference to EF and † indicates a significant difference to OV ($P < 0.05$).

Quadriceps MVIF, FDI muscle MVIF and CMJA height were all not significantly different between phases of the menstrual cycle (all $P > 0.05$; all $d < 0.2$) and OC cycle (all $P > 0.05$; all $d < 0.2$, Table 6.2). The mean percentage changes between phases of the menstrual and OC cycle are presented in Table 6.3. Oestradiol concentrations were not correlated to any measure of muscle function at any time point for either group (all $P > 0.05$).

Table 6.2. Mean ± 1SD muscle force measures in eumenorrheic participants in the early follicular (EF), ovulatory (OV) and mid-luteal (ML) phase and oral contraceptive users at first (PC1) and second (PC2) pill consumption time points and during the pill-free interval (PFI).

	Eumenorrheic			Oral contraceptive		
	EF	**OV**	**ML**	**PC1**	**PC2**	**PFI**
Quadriceps muscle force (N)	391.0 ± 90.8	381.5 ± 95.3	376.7 ± 84.1	396.2 ± 80.7	408.5 ± 85.3	410.1 ± 92.2
First dorsal interosseus muscle force (N)	27.2 ± 6.3	27.2 ± 6.5	26.9 ± 7.0	27.9 ± 3.3	28.4 ± 3.5	28.2 ± 3.9
Countermovement jump with arms height (cm)	26.0 ± 5.3	25.5 ± 5.7	26.3 ± 5.8	30.9 ± 6.6	30.1 ± 4.7	30.2 ± 4.9

Table 6.3. Mean percentage change in muscle force production between the early follicular (EF), ovulatory (OV) and mid-luteal (ML) phases in eumenorrheic females and between the first (PC1) and second (PC2) pill consumption time points and during the pill-free interval (PFI) in oral contraceptive users. N.B. the reference phase for the percentage difference calculation is the second-mentioned phase *e.g.,* where 'EF vs. OV' is +2.5%, this states that mean EF values are 2.5% higher than those in OV.

	Eumenorrheic			Oral Contraceptive		
	EF vs. OV	**EF vs. ML**	**OV vs. ML**	**PC1 vs. PC2**	**PC1 vs. PFI**	**PC2 vs. PFI**
Quadriceps maximum force (%)	+2.5	+3.8	+1.2	-3.0	-3.4	-0.4
First dorsal interosseous muscle force (%)	-0.0	+1.2	+1.2	-1.8	-1.2	0.7
Countermovement jump with arms (%)	+1.3	-1.0	-2.8	+2.6	+2.3	-0.2

6.4. Discussion

This study has shown that, in recreationally active women, muscle force production is not influenced by changes in reproductive hormone profiles during the menstrual cycle, or during different phases of a combined OC cycle, the two most prevalent models of reproductive function in athletes (Chapter 5). This study has used reliable measures of muscle function (Chapter 4; Martin, Cooper, Sale, Compton, & Elliott-Sale, 2015), controlled pre-test diet and activity levels, confirmed hormone concentrations using assays, is adequately powered as calculated using an *a priori* power analysis and used a homogenous OC group. This is the first study to combine these gold-standard practices, and as such, has improved upon previous research in this area and has provided high-quality evidence that muscle force is unaffected by reproductive hormones. This has been demonstrated in the dynamic and isometric force of the quadriceps, a large muscle group with a practical application to sporting performance (Suchomel, Nimphius, & Stone, 2016) and the isometric force of the FDI muscle, a tightly controlled muscle that is singularly involved in abduction of the index finger (Carpentier, Duchateau, & Hainaut, 2001).

The unchanged quadriceps and FDI muscle MVIF is thus supportive of those prior studies showing no difference in muscle force production across the menstrual cycle (Elliott et al., 2005; Elliott, Cable, Reilly, & Diver, 2003; Fridén et al., 2003; Gür, 1997; Janse de Jonge, Boot, Thom, Ruell, & Thompson, 2001; Lebrun, McKenzie, Prior, & Taunton, 1995), although we have been able to decisively show this given our tighter experimental control. Previous studies that have shown differences in muscle force production across the menstrual cycle have not measured hormone concentrations and, as such, were unable to confirm menstrual cycle phase (Bambaeichi et al., 2004; Sarwar et al., 1996; Tenan et al., 2016), not confirmed ovulation (Sarwar et al., 1996), and/or poorly defined the phases of the menstrual cycle (Phillips et al., 1996). Furthermore, Ekenros et al. (2013) showed that knee extensor strength was significantly higher in the luteal phase compared to the follicular phase, although this may have been the result of a learning effect since all participants completed conditions in the same order and similar learning effects were observed in OC-using participants. The current study; used clearly defined menstrual cycle phases that were confirmed with hormonal analyses and controlled extraneous factors that may influence muscle force production such as diet and activity. This well-controlled study supports the majority of research showing that muscle force production is not influenced by the menstrual cycle and is further supported by previous research from our group showing that even supra-physiological changes in reproductive hormone concentrations during in-vitro fertilisation treatment do not influence muscle force production (Elliott et al., 2005).

Muscle isometric force production was also not different between phases of an OC cycle. These results are in line with previous research, which showed unchanged muscle force production at different stages of OC use (Ekenros et al., 2013; Elliott et al., 2005; Nicolay et al., 2008; Sarwar et al., 1996). Previous research has used non-homogenous pill groups, which results in large inter-individual variability (Elliott-Sale et al., 2013) and the possibility of incurring type II errors. In the present study, participants used the same OC preparation, containing the same steroid hormone content (30 μg ethinyl oestradiol; 150 mg Levonorgestrel) with a standardised timing of pill consumption, however, despite these controls the inter-individuality variability in endogenous oestradiol was still higher (PC1: 50.2 ± 47.5; PC2: 27.9 ± 16.8; PFI: 63.7 ± 54.2; all mean ± 1SD pmol·L^{-1}) than previous oestradiol concentrations in homogenous groups (Elliott-Sale et al., 2013). Testing was conducted at the start (days 2-3) and towards the end (days 15-16) of pill consumption and during the PFI (days 3-4) as these represent slightly different hormonal profiles; endogenous oestradiol concentrations increase slightly during the PFI, remain somewhat *elevated* during the start of pill consumption, and are *reduced* after several weeks of OC use (van Heusden & Fauser, 1999). Two-thirds of our blood samples were collected during these *elevated* oestradiol phases (PC1 and PFI), whereas, in the Elliott et al. (2013) study, samples were randomly collected at any point throughout the pill consumption phase. During these *elevated* phases, more individual variation in endogenous oestradiol concentrations was shown, with the standard deviations being 2.8 and 3.2 times higher at PC1 and PFI compared to PC2, reflecting a less stable hormonal milieu. An indeterminable number of samples were collected during the *elevated* oestrogen phases by Elliott et al. (2013), and if this number were low, this could have conceivably resulted in lower inter-individual variability and may account for the difference between the two studies. Therefore, future studies should employ a homogenous pill group, but carefully consider the timing of the samples within either the pill consumption or pill withdrawal phases depending on the research question.

Countermovement jump height with arms was not different across the OC cycle, which is similar to previous findings using various dynamic performance tests (Ekenros et al., 2013; Giacomoni, Bernard, Gavarry, Altare, & Falgairette, 2000b; Rechichi & Dawson, 2009). In contrast, Rechichi & Dawson, (2009) showed that a different type of dynamic movement, reactive strength from a drop jump, varied across an OC cycle. Reactive strength from a 30 cm drop was significantly lower during the late PFI compared to the early PFI and pill consumption, whereas reactive strength from a 45 cm drop was significantly lower in the early and late PFI compared to pill consumption (Rechichi & Dawson, 2009). The authors suggested that these results may be explained by a rise in endogenous oestradiol concentration in the late

PFI, as previous research has shown neuromuscular control during a drop jump is affected by changing reproductive hormones across a menstrual cycle (Dedrick et al., 2008). Despite this, the apparently conflicting results between 30cm and 45cm drop reactive strength are not immediately explainable and may indicate the occurrence of type I errors. The current study measured CMJA height in the middle of the PFI, where endogenous oestradiol concentration was slightly, although not significantly, elevated when compared to PC2 ($d = 1.01$, $P = 0.075$), however vertical jump height was not different between time points.

Menstrual cycle phase also had no effect on CMJA height, which is in line with some (Ekenros et al., 2013; Giacomoni et al., 2000) but not all previous research examining dynamic movements across a menstrual cycle (Davies et al., 1991a). Ekenros et al. (2013) showed that 1-leg hop performance did not change between menstrual cycle phases in 17 women, while Giacomoni et al. (2000) did not show any differences in vertical jump height in seven eumenorrheic women. These studies used hormonal assays to confirm menstrual cycle phase, which were not employed by Davies et al. (1991a). The sum of this evidence would suggest that jump performance is not affected by the menstrual cycle.

In conclusion, this study has shown that neither isometric nor dynamic muscle force production is significantly influenced by menstrual cycle phase or OC pill use (all $P > 0.05$). Moreover, the effect sizes between phases of the menstrual and OC cycle were trivial for all muscle force measures (all $d < 0.2$). In the RED-S model, it is suggested that muscle force may be reduced with low energy availability (Mountjoy et al., 2014), however it is unclear whether a down-regulation of reproductive hormones due to low energy availability will synergistically affect muscle strength, as seen with bone metabolism (De Souza et al., 2008). This study has shown that, independent of changes in energy availability, there is no effect of circulating reproductive hormone concentrations on muscle strength and this is not an important consideration for practitioners. Future research is now required to assess whether the reproductive hormone environment influences the muscle function response to low energy availability (see Chapter 9).

Chapter 7.0. The effects of menstrual cycle phase and oral contraceptive use on cognitive function

7.1. Introduction

Reproductive hormones play an important role in the maintenance of brain structure and function, evidenced for example by the decline in cognition following the menopause, which can be ameliorated with HRT (Markou, Duka, & Prelevic, 2005; van Amelsvoort, Compton, & Murphy, 2001). This may have implications for female athletes, as oestrogen and progesterone concentrations are down-regulated in response to low energy availability, altering the endogenous hormonal milieu (Loucks et al., 1998). The RED-S model suggests that aspects of cognitive function may be affected by reduced energy availability, however it is unclear whether the alterations to reproductive hormone concentrations, such as those that occur during the menstrual cycle and with OC use, may impact cognitive function independent of the energy-related effects (De Souza et al., 2014; Mountjoy et al., 2014). The effects of reduced energy availability on cognitive function will be studied in Chapter 10, however the aim of this Chapter was to understand the effects of changes in reproductive hormones, independent of changes in energy availability, during the menstrual cycle and OC cycle, which are the two most common reproductive hormone profiles in female athletes (Chapter 5).

Several studies have showed that performance in male-dominated tasks, such as the mental rotation test, is improved in early follicular phase of the menstrual cycle when reproductive hormone concentrations are low (Courvoisier et al., 2013; Hampson, 1990; Hausmann et al., 2000; Maki et al., 2002). In contrast, performance in female-dominated tasks such as verbal fluency and verbal memory, has been shown to be superior in the mid-luteal phase when reproductive hormone concentrations are high (Hampson, 1990; Maki et al., 2002; Rosenberg & Park, 2002). Despite this, the majority of studies have demonstrated that there is no difference in performance in these components of cognitive function between phases of the menstrual cycle (Epting & Overman, 1998; Gordon & Lee, 1993; Griksiene & Ruksenas, 2011; Mordecai et al., 2008; Phillips & Sherwin, 1992).

There are, however, methodological issues with previous research that limits the ability to clearly identify the effects of reproductive hormones on cognitive performance. Previous studies have typically only assessed cognitive performance at two phases of the menstrual cycle; the early follicular phase and the mid-luteal phase. This may not be able to adequately differentiate the effects of oestrogen and progesterone on cognitive performance as these phases represent low oestrogen and low progesterone (early follicular), and high oestrogen and high progesterone (mid-luteal), and oestrogen and progesterone are known to often exert contrasting effects (Chen & Jow, 1989). Studies that have attempted to overcome this and measure mental rotation, verbal fluency or verbal memory at time points when oestrogen

concentrations are high and progesterone are low (late-follicular/ovulatory) have relied upon predicting the time point of peak oestrogen concentrations based upon monitoring of previous cycles (Gordon & Lee, 1993; Solís-Ortiz & Corsi-Cabrera, 2008; Jacobs & D'Esposito, 2011), which may not accurately estimate hormone concentrations in the 'tested' cycle (Fehring et al., 2006). Testing in the period following the LH surge, as detected by ovulation test kits, presents a relatively high (mean 320.6 pmol·L^{-1}; Stricker et al., 2006) oestrogen concentration, low progesterone concentration, and is not reliant on predicting menstrual cycle phase length so may be a more suitable time point. Few studies have confirmed ovulation (Sundstrom Poromaa & Gingnell, 2014), which is necessary as oestrogen and progesterone concentrations are reduced in anovulatory cycles and are therefore not representative of a typical menstrual cycle (Hambridge et al., 2013). Furthermore, no studies to date have controlled diet and exercise in the lead in to test sessions, which are known to influence cognitive function (Kamijo, Nishihira, Higashiura, & Kuroiwa, 2007; Nehlig, 2010).

The effects of OC use on cognitive function have been studied on fewer occasions than the menstrual cycle. Some studies comparing pill consumption to the PFI have shown no effects on verbal fluency, verbal memory and mental rotation performance (Gordon & Lee, 1993; Griksiene & Ruksenas, 2011). In contrast, Mordecai et al. (2008) showed that in 16 OC users, verbal memory performance (number of words recalled) was improved in the pill consumption (Day 13-15 pill consumption) compared to the PFI (Day 2-3) of an OC cycle, suggesting a positive effect of exogenous reproductive hormones on verbal memory, while there was no difference in verbal fluency, visual memory, spatial ability or attention between OC phases. These studies all used mixed-pill groups, providing different types and concentrations of synthetic hormones, which results in a larger inter-individual variation in endogenous reproductive hormone concentrations (Elliott-Sale et al., 2013), and previous research has shown that the type of progestin used influences the cognitive response (Gogos, 2013; Wharton et al., 2008). Furthermore, these studies have not standardised the time of day of pill consumption in relation to cognitive performance testing, which may have a significant effect as synthetic oestrogens and progestins display typical pharmacokinetics of orally administered drugs, in that concentrations rapidly reach a peak within 60-90 min, and then decline in a curvilinear manner (Carol et al., 1992).

The focus on sexually dimorphic tasks has meant that cognitive domains such as executive function, attention and perceptual-motor performance have not been well-studied across the menstrual or OC cycle. The majority of studies assessing these cognitive domains have not measured reproductive hormones to confirm menstrual cycle phase (Broverman et al., 1981; Farrar et al., 2015; Hampson, 1990; Symonds et al., 2004; Upadhayay & Guragain, 2014),

measured performance in only two phases of the menstrual cycle, so are unable to differentiate the effects of oestrogen and progesterone (Broverman et al., 1981; Farrar et al., 2015; Hatta & Nagaya, 2009; Solis et al., 2015), or used heterogeneous OC groups (Griksiene & Ruksenas, 2009; Mordecai et al., 2008). In particular, vigilance has not been well researched as previous studies have either assessed visuospatial attention (Brötzner et al., 2015), where performance may be mediated by the effects of reproductive hormones on the spatial component of the task (Hampson, 1990), or a short (1 min) visual scanning task (Griksiene & Ruksenas, 2009), which assesses visual scanning performance and does not require sustained attention. Furthermore, while simple response time has been shown to not vary across the menstrual cycle in a well-controlled study (Griksiene & Ruksenas, 2009), response times to more complex stimuli have not been well-studied across the menstrual cycle or OC cycle.

Therefore, there is a need for further research to clarify the effects of changes in reproductive hormones across the menstrual cycle or OC cycle, improving previous research by tightly controlling cycle phases and pre-trial requirements, in addition to measuring performance in cognitive domains and tests that have not been fully explored. The aim of this study was to assess whether cognitive performance changes across the menstrual cycle or OC cycle in a range of cognitive tasks, independent of changes in energy availability.

7.2. Methods

7.2.1 Participants

The participant population was described Section 6.2.1 and all participants were English speakers.

7.2.2 Experimental design

The experimental design was described in Section 6.2.1 and 6.2.2. Testing sessions were conducted in the order in which the participants presented each condition. For example, eumenorrheic participants starting the main experimental trials after expected ovulation within their current cycle would wait until the next identifiable time point of the menstrual cycle (i.e. menstruation) to arrange the EF test session. Ovulatory and ML phase test sessions would then be arranged once ovulation was confirmed using LH test kits. In the eumenorrheic participants, the inability to detect ovulation in some cycles, as is commonly seen in eumenorrheic women (Prior et al., 2015), combined with practical scheduling issues of arranging participants to attend the laboratory with 24 h notice (post LH surge) at a fixed time of day, resulted in a non-counterbalanced study design (See Table 7.1). The testing of OC users in the chronological

order that they were able to complete test sessions also resulted in a non-counterbalanced design (Table 7.1)

Table 7.1. Number of participants that completed each phase as their 1st, 2nd or 3rd experimental trial for eumenorrheic (Early Follicular, EF; Ovulatory, OV; Mid-luteal, ML) and oral contraceptive users (pill consumption 1, PC1; pill consumption 2, PC2; pill-free interval, PFI).

Phase	Session		
Eumenorrheic	1st session (n)	2nd session (n)	3rd session (n)
EF	11	3	0
OV	0	9	5
ML	3	2	9
Oral contraceptive	1st session (n)	2nd session (n)	3rd session (n)
PC1	4	3	7
PC2	7	3	4
PFI	3	8	3

7.2.3 Experimental protocol

Upon arrival to the laboratory, height and body mass were measured (Section 3.3.1), before 30 ml of blood was drawn from an antecubital vein for measurement of serum oestradiol (Section 3.8). Participants were then rested for 30 min before being provided a breakfast consisting of cereal, milk, toasted bread and butter (1g·kgBM^{-1} carbohydrate, 0.1g·kgBM^{-1} fat and 0.19g·kgBM^{-1} protein), which they were asked to consume within 20 min. Participants then completed the POMS (Section 3.7.1) and PSQI questionnaires (Section 3.7.2), followed by completion of the cognitive test battery in the following order:

- RAVLT (Section 3.6.2)
- Mental rotation test (Section 3.6.3)
- Verbal fluency test (Section 3.6.4)
- Visual search test (Section 3.6.5)
- Stroop task (Section 3.6.6)
- RVIP (Section 3.6.7)

The cognitive function tests were standardised using the protocols outlined previously (Section 3.6.1).

7.2.4 Statistical analysis

An *a priori* power analysis indicated that 11 participants in each group were required for a study power of 80% (1 – β = 0.8) and 16 participants in each group were required for a study power of 95% (1- β = 0.95), at an alpha level of $P < 0.05$, based upon mental rotation scores from previous research (Hooven et al. 2010). Data for EU participants and OC users were analysed separately. All data were checked for normality using the Shapiro-Wilk test. Due to the non-counterbalanced study design, data were first checked for learning effects by organising test sessions in to chronological order and analysed using one-way repeated measures ANOVA (RAVLT, verbal fluency test, RVIP) and two-way (order x level) repeated measures ANOVA (mental rotation test, visual search test, Stroop test). Where significant learning effects were evident, data were not analysed further due to an inability to determine effects due to menstrual cycle/OC phase from the learning effects. Where there was no learning effect, data were analysed using one-way repeated measures ANOVA (RAVLT, RVIP, POMS, PSQI) and two-way (time x level) repeated measures ANOVA (mental rotation test, visual search test, Stroop test) with significant effects explored using Bonferroni adjusted t-tests. Effect sizes were calculated using Cohen's d (Cohen & Jacob, 1992) and were described as trivial (0.0 – 0.19), small (0.20 – 0.49), medium (0.50 – 0.79) and large (> 0.80). Data are presented as mean ± 1SD and the level of significance was set at $P \leq 0.05$.

7.3. Results

7.3.1. Learning Effect Analyses

Significance values for cognitive measures analysed in chronological order of test session for a learning effect are presented in Table 7.2. Significant main effects of order ($P < 0.05$) or order x level interaction effects ($P < 0.05$), indicative of a learning effect, were evident for RAVLT acquisition and learning rate, mental rotation test response time, verbal fluency words produced and Stroop test response time in eumenorrheic participants. In OC users, significant main effects of time ($P < 0.05$) were evident for RAVLT acquisition, mental rotation test response time and Stroop test response time. Therefore, no further analyses were conducted on these data.

7.3.2. Menstrual cycle and oral contraceptive use analyses

7.3.2.1. Oestradiol

For eumenorrheic participants, oestradiol concentrations were significantly different between menstrual cycle phases (main effect time; $P < 0.05$). EF phase oestradiol concentrations were significantly lower than OV ($P = 0.02$; d = 1.18) and ML phases ($P < 0.001$; d = 1.97) and ML phase oestradiol concentrations were significantly higher than the OV phase ($P = 0.03$; d =

0.76). For OC users, there was no significant effect of OC phase on oestradiol concentrations (P = 0.076), but there was a medium effect size when comparing PC1 to PC2 (d = 0.69, P = 0.25) and a large effect size when comparing PC2 to the PFI (d = 1.01, P = 0.075). These data are presented in Chapter 6 (Figure 6.1).

7.3.2.2. Rey Auditory Verbal Learning Test

Proactive interference, retroactive interference and forgetting were not different between menstrual cycle phases and OC phases (main effect time, all P > 0.05). Learning rate was not analysed in eumenorrheic women and was not different between OC phases (main effect time, P = 0.250).

7.3.2.3. Mental rotation test

Accuracy was not different between menstrual cycle phases or OC phases (main effect time, both P > 0.05). Accuracy was reduced with increasing rotation angle for both groups (main effect level, both P < 0.001), although menstrual cycle phase or OC phase did not influence the accuracy across angles of rotation (time x level interaction effect, both P > 0.05).

7.3.2.4. Verbal fluency

Words produced in the verbal fluency test was not analysed in eumenorrheic women and was not different between OC phases (main effect time, P = 0.336).

7.3.2.5. Stroop test

Accuracy was not different between menstrual cycle phases or OC phases (main effect time, all P > 0.05). Accuracy was greater in the simple level compared to the complex level for both groups (main effect level, P < 0.05), although menstrual cycle phase and OC phase did not influence accuracy across test levels (time x level interaction effect, all P > 0.05).

7.3.2.6. Rapid Visual Information Processing task

Response time, true positive rate and miss rate were not different between menstrual cycle phases or OC phases (main effect time, P > 0.05).

7.3.2.7. Mood and Sleep

Pittsburgh Sleep Questionnaire Index scores and all dimensions of the POMS were not significantly different between menstrual cycle phases (all P > 0.05) or OC cycle phases (all P > 0.05).

Table 7.2. Significance values for cognitive function test measures, when analysed for a learning effect in chronological order of session completion.

Test/component	Eumenorrheic		Oral Contraceptive	
	Main effect of Order	Order x Level Interaction effect	Main effect of Order	Order x Level Interaction effect
RAVLT				
Acquisition	0.016*	-	0.012*	-
Learning rate	0.004*	-	0.623	-
Proactive interference	0.140	-	0.198	-
Retroactive interference	0.310	-	0.971	-
Forgetting	0.402	-	0.837	-
Mental rotation test				
Response time	< 0.001*	0.054	0.012*	0.09
Accuracy	0.247	0.785	0.414	0.653
Verbal fluency test				
Word produced	0.018*	-	0.346	-
Visual search test				
Response time	0.504	0.343	0.585	0.370
Accuracy	0.866	0.540	0.830	0.634
Stroop test				
Response time	0.019*	0.019*	0.027*	0.836
Accuracy	0.204	0.065	0.927	0.447
RVIP				
Response time	0.813	-	0.070	-
True positive rate	0.177	-	0.383	-
Miss rate	0.067	-	0.987	-

Rey Auditory Verbal Learning Test, RAVLT; Rapid Visual Information Processing Task, RVIP

Table 7.3. Cognitive function absolute values for eumenorrheic participants in the early follicular (EF), ovulatory (OV) and mid luteal (ML) phases and oral contraceptive users in the early pill consumption (PC1), late pill consumption (PC2) and pill-free interval (PFI) phases.

	Eumenorrheic			Oral contraceptive		
	EF	OV	ML	PC1	PC2	PFI
RAVLT						
Acquisition	-	-	-	78.0 ± 27.6	84.6 ± 27.1	81.3 ± 28.0
Learning rate	-	-	-	4.4 ± 4.0	5.9 ± 2.4	5.7 ± 2.4
Proactive interference	1.0 ± 1.9	1.9 ± 2.3	1.2 ± 1.9	0.6 ± 2.4	1.3 ± 2.1	1.1 ± 2.3
Retroactive interference	2.1 ± 2.0	1.5 ± 2.1	2.0 ± 2.0	0.7 ± 3.2	0.9 ± 1.4	1.1 ± 1.1
Forgetting	2.1 ± 1.5	2.2 ± 1.7	2.9 ± 1.9	0.7 ± 3.2	1.4 ± 1.8	1.6 ±1.5
Mental rotation test						
0° RT (ms)	-	-	-	-	-	-
0° Accuracy	0.94 ± 0.06	0.90 ± 0.11	0.89 ± 0.17	0.95 ± 0.05	0.94 ± 0.07	0.95 ± 0.07
20° RT (ms)	-	-	-	-	-	-
20° Accuracy	0.93 ± 0.07	0.91 ± 0.11	0.91 ± 0.13	0.89 ± 0.09	0.90 ± 0.11	0.93 ± 0.13
40° RT (ms)	-	-	-	-	-	-
40° Accuracy	0.83 ± 0.18	0.88 ± 0.13	0.91 ± 0.10	0.84 ± 0.14	0.80 ± 0.16	0.85 ± 0.20
60° RT (ms)	-	-	-	-	-	-
60° Accuracy	0.79 ± 0.17	0.80 ± 0.15	0.81 ± 0.19	0.74 ± 0.23	0.69 ± 0.21	0.74 ± 0.21
80° RT (ms)	-	-	-	-	-	-
80° Accuracy	0.76 ± 0.18	0.75 ± 0.21	0.81 ± 0.19	0.75 ± 0.19	0.65 ± 0.19	0.69 ± 0.20
Overall RT (ms)	-	-	-	-	-	-
Overall Accuracy	0.85 ± 0.10	0.82 ± 0.11	0.86 ± 0.12	0.83 ± 0.11	0.80 ± 0.11	0.83 ± 0.12
Verbal fluency test						
Words produced	-	-	-	-	-	
Visual Search						
Simple RT (ms)	517 ± 23	516 ± 15	511 ± 29	517 ± 41	527 ± 34	508 ± 38
Simple Accuracy	0.98 ± 0.04	0.98 ± 0.03	0.99 ± 0.02	0.99 ± 0.02	0.99 ± 0.03	0.99 ± 0.02
Complex RT (ms)	1596 ± 157	1616 ± 247	1659 ± 239	1518 ± 244	1496 ± 274	1582 ± 256
Complex Accuracy	0.99 ± 0.01	1.00 ± 0.01	0.99 ± 0.01	0.99 ± 0.02	0.97 ± 0.04	0.98 ± 0.03
Stroop test				40.8 ± 0.8	41.1 ± 9.7	38.1 ± 8.1
Simple RT (ms)	-	-	-	-	-	-
Simple Accuracy	0.99 ± 0.03	0.97 ± 0.05	0.98 ± 0.04	0.98 ± 0.04	0.99 ± 0.03	1.00 ± 0.01
Complex RT (ms)	-	-	-	-	-	-
Complex Accuracy	0.97 ± 0.05	0.97 ± 0.04	0.95 ± 0.04	0.97 ± 0.03	0.96 ± 0.03	0.96 ± 0.04
RVIP						
RT (ms)	546 ± 87	524 ± 95	533 ± 96	483 ± 70	531 ± 94	513 ± 58
True positive rate	0.77 ± 0.18	0.81 ± 0.16	0.81 ± 0.15	0.79 ± 0.20	0.74 ± 0.25	0.69 ± 0.25
Miss rate	0.43 ± 0.14	0.39 ± 0.16	0.41 ± 0.13	0.42 ± 0.20	0.40 ± 0.17	0.42 ± 0.25

Rapid Visual Information Processing, RVIP; Response time, RT; Rey Auditory Verbal Learning Test, RAVLT

7.4. Discussion

Similar to the results observed in Chapter 6 for muscle function, there were no differences in cognitive function between phases of the menstrual cycle or OC use for any of the analysed cognitive function tests, despite oestradiol concentrations being significantly different between menstrual cycle phases. This is the first study to test cognitive performance across an OC cycle in participants using the same OC brand (Microgynon®), which is the most representative of the female athlete population (Chapter 5).

Our findings are in contrast to some, but not all previous research, that has shown mental rotation performance, a male-dominated task, is superior in the early follicular phase, when oestrogen concentrations are lowest (Courvoisier et al., 2013; Hausmann et al., 2000; Maki et al., 2002). We were unable to analyse mental rotation response time, as this element displayed a learning effect, although we showed mental rotation test accuracy was unaffected by menstrual cycle phase, which is the component that has previously been shown to vary across the menstrual cycle (Courvoisier et al., 2013; Hausmann et al., 2000; Maki et al., 2002). These studies measured performance daily for 8 weeks, which may confound interpretation due to learning effects (Courvoisier et al., 2013), or only measured two phases of the menstrual cycle, limiting the ability to study the effects of reproductive hormones (Hausmann et al., 2000; Maki et al., 2002). Therefore, the current study has improved on these elements of study design. There was also no effect of OC phase on mental rotation performance, which is similar to previous research (Gordon & Lee, 1993; Griksiene & Ruksenas, 2011; Mordecai et al., 2008), although the present study employed a stronger study design by using a homogenous pill group, thus reducing inter-participant variability, which is especially important in studies of cognition (Gogos, 2013; Wharton et al., 2008).

The present study was unable to determine whether verbal fluency varied between menstrual cycle phases, although we showed verbal fluency was not different between phases of an OC cycle, similar to other observations (Griksiene & Ruksenas, 2011; Mordecai et al., 2008). Verbal memory has been reported to vary across the menstrual cycle (Maki et al., 2002; Rosenberg & Park, 2002), while others have shown no effects (Jacobs & D'Esposito, 2011; Mordecai et al., 2008). For the RAVLT, the current study showed that learning rate, proactive interference, retroactive interference and delayed recall were unaffected by menstrual cycle phase. Mordecai et al. (2008) showed that verbal memory, as measured using the California Verbal Leaning Test (CVLT), was different between OC phases; total words recalled across 5 learning trials, words recalled after a short delay and words recalled after a long delay were improved on pill consumption days compared to the PFI. The CVLT is similar to the RAVLT,

asking participants to recall 16 words from 4 semantic categories in comparison to the RAVLT which has 15 unrelated words, and both produce similar outcome measures (Stallings, Boake, & Sherer, 1995). Whilst we were unable to determine whether total words recalled over the 5 trials (acquisition) was different between phases of the menstrual cycle, we showed that retroactive interference and forgetting were not different between phases, in contrast to Mordecai et al. (2008). These opposing results may be a result of the difference in test difficulty between the RAVLT and CVLT, in which performance in the CVLT is typically higher due to the use of semantic clustering strategies for similar words (Crossen & Wiens, 1994). It has been suggested that females outperform males in word recall tasks due to improved semantic clustering ability (Andreano & Cahill, 2009), therefore the lack of differences observed in the current study may be due to the reduced capacity for semantically relating words in the RAVLT compared to the CVLT, limiting any potential reproductive hormone mediated effects.

Accuracy on the Stroop test was also unchanged across the menstrual cycle or OC cycle. Previous research has shown that the time taken to complete the Stroop word-colour list was superior in the follicular phase (Upadhayay & Guragain, 2014), and whilst we were unable to determine speed, we showed that accuracy was not changed between phases, in contrast to Hatta & Nagaya, (2009), who showed improved accuracy in the luteal phase. Stroop test performance is often categorised as a measure of executive function (shifting), however can also be classified as a measure of selective attention. Various measures of attention have previously been studied, showing no changes across the menstrual cycle (Griksiene & Ruksenas, 2009; Mordecai et al., 2008) or OC cycle (Griksiene & Ruksenas, 2009; Mordecai et al., 2008), which was verified in the current study using the RVIP task, which has not previously been studied. Similarly, simple response time, as assessed using the Donders reaction time test, was previously shown to remain unchanged across the menstrual cycle (Griksiene & Ruksenas, 2009) and OC cycle (Griksiene & Ruksenas, 2009). This was also confirmed in the current study using the visual search test simple level, in addition to showing that complex response time, as assessed using the visual search test complex level, was not affected by menstrual cycle or OC phase, which has not previously been studied.

The research design utilised in this study standardised diet and exercise in the lead-in to each session, conducted tests on well-defined phases of the menstrual cycle (confirming ovulation using LH test kits and measuring oestradiol concentrations to confirm phases), and used a homogenous pill group, standardising the time of day of OC consumption. Participants were familiarised to the cognitive test battery prior to the experimental trials; previous research showed that this is sufficient as there is a learning effect from first to second test presentation,

with no further improvements after this (Falleti, Maruff, Collie, & Darby, 2006). In the current study, it was shown that performance was improved over time for several of the cognitive tests, so these could not be used. Ideally, a counter-balanced study design would have been used, however this was impractical due to the constraints of test scheduling. In particular, strictly mandating that participants attend the laboratory the morning after the LH surge was problematic as this was often not possible for participants to re-arrange their schedule at short notice, or anovulatory cycles, which regularly occur spontaneously in otherwise ovulatory women (Prior et al., 2015), were common and particularly delayed ovulatory and mid-luteal phase testing.

We have shown that for a number of cognitive function tasks, performance is not different between phases of the menstrual cycle or OC cycle, the two most common reproductive hormone models in female athletes (Chapter 5). Similar to muscle function (Chapter 6), this shows that the circulating concentrations of reproductive hormones do not influence cognitive function, independent of changes in energy availability, as standardised dietary and exercise practices were used. Researchers and practitioners therefore do not need to consider the acute variation in reproductive hormone concentrations on cognitive performance. Further research is now required (Chapter 10) to assess whether low energy availability affects cognitive performance, as stated in the RED-S model (Mountjoy et al., 2014), and whether the reproductive hormone environment (*e.g.* eumenorrhea or OC use) affects the response to low energy availability.

Chapter 8.0. The effects of menstrual cycle phase and oral contraceptive use on bone metabolism

8.1. Introduction

The Triad and RED-S models state that low energy availability and menstrual dysfunction negatively affect bone health (De Souza et al., 2014; Mountjoy et al., 2014), with much of the evidence presented due to changes in bone metabolism with low energy availability (Ihle & Loucks, 2004; Papageorgiou et al., 2017), hypothalamic amenorrhea (Grinspoon et al., 1999) or a combination of these factors (De Souza et al., 2008). The majority of this research is based upon bone metabolic measurements of non-HC-using women in the follicular phase of the menstrual cycle (De Souza et al., 2008; Ihle & Loucks, 2004; Papageorgiou et al., 2017), with the response to low energy availability not being studied in other menstrual cycle phases. Despite Chapter 5 showing that approximately half of female athletes use HCs, no research has explored how HC use affects the bone metabolic response to low energy availability. In order for future research to accurately assess how different reproductive hormone profiles influence the bone metabolic response to low energy availability, it is important that the changes in bone marker concentrations are known across the menstrual cycle and combined OC cycle, the most prevalent type of HC used (Chapter 5), independent of changes in energy availability.

In eumenorrheic women, P1NP concentrations were 6.4% (Gass et al., 2008) and 11.4% (Liakou et al., 2016) higher in the luteal phase compared to the follicular phase, while β-CTX concentrations were ~9-13% higher in the luteal phase (Gass et al., 2008; Liakou et al., 2016; Mozzanega et al., 2013; Niethammer et al., 2015). The ability to interpret these studies, however, is limited as standardisation procedures recommended by the IOF (Szulc et al., 2017) were not followed; including not restricting exercise in the 24 h before measurements (Gass et al., 2008; Liakou et al., 2016; Mozzanega et al., 2013; Niethammer et al., 2015) and not using fasted measurements or controlling for the time of day appropriately (Mozzanega et al., 2013; Niethammer et al., 2015). Furthermore, populations aged 30-45 years (Liakou et al., 2016) and >40 years (Niethammer et al., 2015) were used, which are not comparable to female athletes (Chapter 5 mean age 24 ± 4 years), two studies (Gass et al., 2008; Niethammer et al., 2015) did not provide details of assays used to measure bone markers and Niethammer et al., (2015) did not clearly define the menstrual cycle phases in which measurements were taken. All of these factors limit the ability to interpret these data. Further research is required to assess P1NP and β-CTX concentrations across the menstrual cycle using standardised procedures recommended by the IOF to reduce pre-analytical variability. The bone formation marker BAP should also be measured to provide a more complete picture of bone metabolism across the menstrual cycle as, unlike P1NP, this is specific to bone (Szulc et al., 2017) and represents mineralisation rather than collagen turnover (Crockett, Rogers, Coxon, Hocking, & Helfrich,

2011). Previous research has shown contrasting results across the menstrual cycle (Chiu et al., 1999; Gass et al., 2008; Nielsen et al., 1990; Niethammer et al., 2015).

In OC users, P1NP has only been assessed across a pill cycle in women that had been using an OC for 2 months, which may result in poor cycle control (Foidart et al., 2000), and that had chronic posterior pelvic pain (Wreje et al., 2000), which may affect collagen metabolism (Kristiansson et al., 1996). No other studies have assessed serum P1NP, BAP or β-CTX across an OC cycle and, therefore, research is required to show whether there are differences between pill consumption phases and the PFI, in a homogenous group of OC users. Therefore, the aim of this study was to examine if there are changes in circulating concentrations of P1NP, BAP and β-CTX across the menstrual cycle and OC cycle.

8.2. Method

8.2.1. Participants

The participants were described in Section 6.2.1.

8.2.2. Experimental design

The experimental design was described in Section 6.2.2.

8.2.3. Experimental protocol

Upon arrival to the laboratory 30 ml of blood was drawn from the antecubital vein for measurement of oestradiol, β-CTX, P1NP and BAP and were analysed as described in Section 3.8.2.

8.2.4. Statistical analysis

Data were checked for normality using the Shapiro-Wilk test. Eumenorrheic and OC participant characteristics were compared using an independent samples t-tests. Oestradiol concentrations and bone metabolic markers were analysed independently for eumenorrheic and OC participants using one-way repeated measures ANOVAs (SPSS v 23.0), with significant effects explored using Bonferroni adjusted t-tests. Where sphericity of data were violated, Greenhouse-Geisser adjustments were used. Between-group comparisons were made using independent samples t-tests on the mean values for each participant calculated across the three phases. Effect sizes were calculated using Cohen's d (Cohen & Jacob, 1992) and were described as trivial (0.0 – 0.19), small (0.20 – 0.49), medium (0.50 – 0.79) and large (> 0.80). Pearson's correlation coefficients were used to cross-correlate oestradiol concentrations and bone metabolic markers for eumenorrheic participants and OC users independently. For

bone metabolism markers, mean % change between different phases of the menstrual cycle or OC cycle were calculated and individual % change responses were characterised by presenting the range of responses in addition to the relative number of participants whose bone marker concentrations increased or decreased between phases. Data are presented as mean $\pm$ 1SD and the level of significance was set at $P \leq 0.05$.

8.3. Results

8.3.1. Between group comparisons

Mean oestradiol concentrations were significantly ($P < 0.001$; $d = 3.05$) higher in eumenorrheic participants (367.4 ± 182.3 pmol·L^{-1}) compared to OC users (47.3 ± 27.4 pmol·L^{-1}). There were no differences between eumenorrheic and OC groups for β-CTX (0.56 ± 0.18 vs. 0.50 ± 0.20 ng·mL^{-1}; $P = 0.37$; $d = 0.32$), P1NP (64.9 ± 21.9 vs. 62.9 ± 22.1 ng·mL^{-1}; $P = 0.81$; $d = 0.03$) and BAP (18.9 ± 5.4 vs. 17.6 ± 3.8 IU·L^{-1}; $P = 0.47$; $d = 0.27$; Figure 8.1).
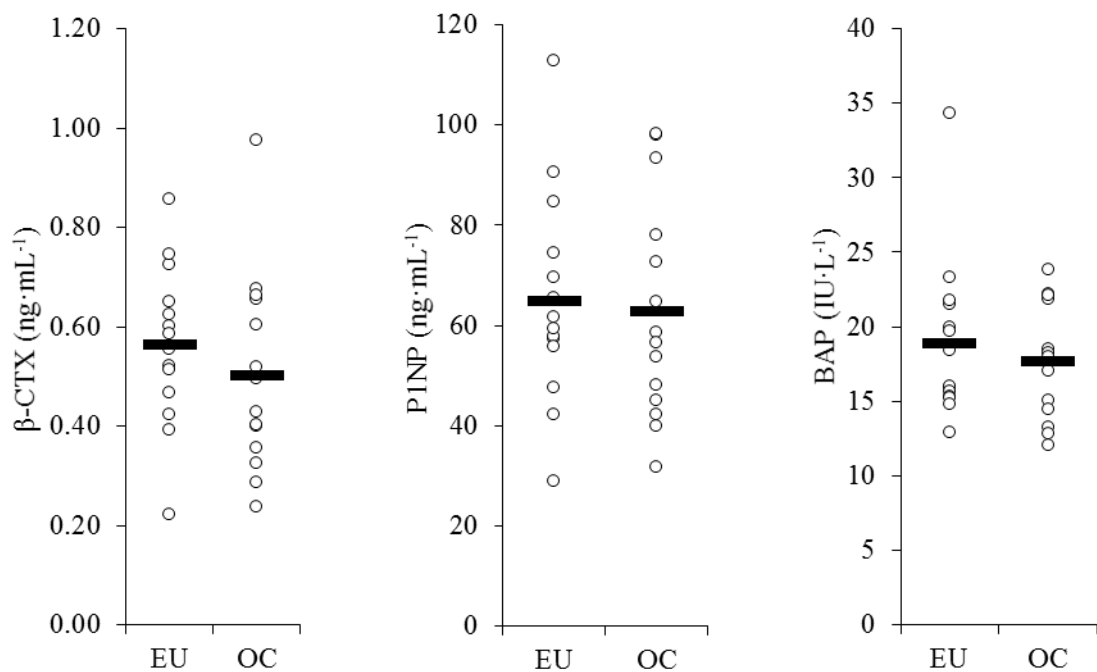
Figure 8.1. Univariate scatter plots with individual data points and mean values for eumenorrheic (EU) participants and oral contraceptive (OC) users mean values across all phases measured for Carboxy-terminal cross-linking telopeptide of type 1 collagen (β-CTX), Procollagen type 1 N telopeptide (P1NP) and bone-specific alkaline phosphatase (BAP) concentrations.

8.3.2. Within-group comparisons

8.3.2.1. Oestradiol

For eumenorrheic participants, EF phase oestradiol concentrations were significantly lower than OV (P = 0.02; d = 1.18) and ML phases (P < 0.001; d = 1.97) and ML phase oestradiol concentrations were significantly higher than the OV phase (P = 0.03; d = 0.76). For OC users, there was no significant effect of OC phase on oestradiol concentrations (P = 0.076), but there was a medium effect size when comparing PC1 to PC2 (d = 0.69, P = 0.25) and a large effect size when comparing PC2 to the PFI (d = 1.01, P = 0.075). These data are presented in Figure 6.1.

8.3.2.2. Carboxy-terminal cross-linking telopeptide of type 1 collagen

For eumenorrheic participants, there was no main effect of menstrual cycle phase (P = 0.632) for β-CTX concentrations. For OC users, β-CTX concentrations were significantly different between different pill consumption phases (P = 0.006; Figure 8.2). Compared to PC2, β-CTX concentrations were significantly higher at PC1 (16.0%; P = 0.015; d = 0.37) and were 14.7% higher at PFI, however this was not significantly different (P = 0.065; d = 0.35). Mean percentage differences between menstrual cycle and OC phases are shown in Table 8.1.

In the eumenorrheic group, 8/14 participant's β-CTX concentrations were higher in the EF phase compared to the OV phase, with differences between phases ranging from +42.3% to -62.4%, and 8/14 were higher in the EF phase compared to the ML phase, ranging from +33.6% to -21.2%. In the OC group, 12/14 OC-using participant's β-CTX concentrations were reduced from PC1 to PC2, ranging from -30.7% to +12.1%, and 11/14 OC participant's β-CTX concentrations were lower in PC2 compared to PFI, ranging from -40.4% to + 7.2%.

8.3.2.3. Procollagen type 1 N telopeptide

There was no effect of phase for eumenorrheic (P = 0.074) and OC participants (P = 0.096; Figure 8.3) for P1NP and mean percentage differences between phases are shown in Table 8.1.

In the eumenorrheic group, 10/14 participant's P1NP concentrations were increased from the OV phase to the ML phase, with the differences between phases ranging from -8.4% to +52.7% and with 6 participant's P1NP concentrations increasing by > 25%. In the OC group, 12/14 participant's P1NP concentrations increased from PC1 to PC2, with the differences ranging from -8.1% to +70.8%.

8.3.2.4. Bone-specific alkaline phosphatase

There was no significant effect of phase for eumenorrheic (P = 0.588) and OC participants (P = 0.602; Figure 8.4) for BAP and mean percentage differences between phases are shown in Table 8.1.

In the eumenorrheic group, 7/14 eumenorrheic participant's BAP concentrations were reduced from EF to OV, ranging from -42% to + 37.2%, and 8/14 EU participant's BAP concentrations were reduced from EF phase to ML phase, ranging from -42.1% to +26.2%. In the OC group, 7/14 participant's BAP concentrations were reduced from PC1 to PC2, with differences ranging from -49.1% to -56.7%, and 9/14 participant's BAP concentrations were reduced from PC1 to PFI, ranging from -31.5% to +27.8%.
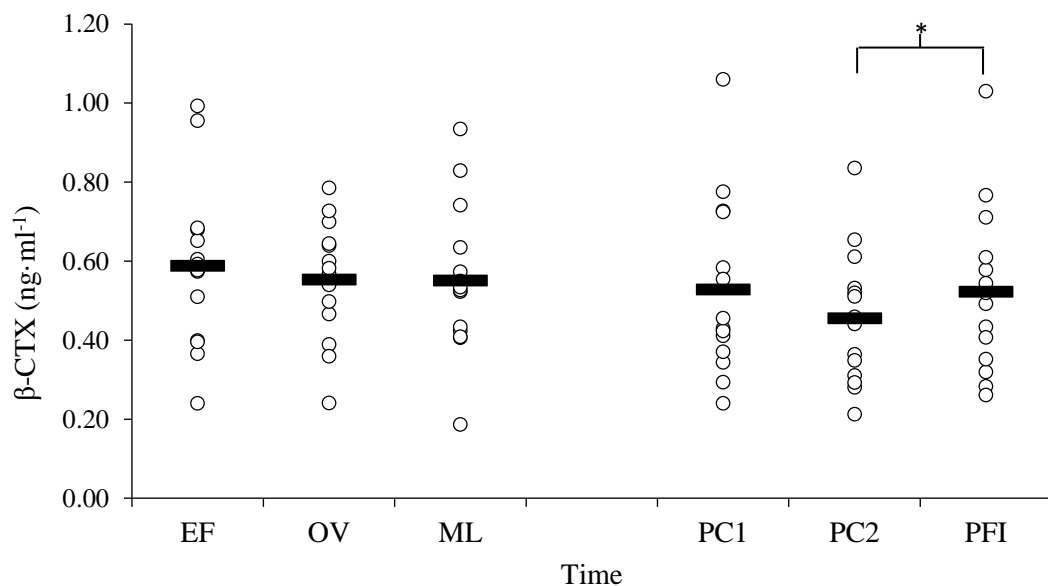
Figure 8.2. Univariate scatter plots with individual data points and mean values for Carboxy-terminal cross-linking telopeptide of type 1 collagen (β-CTX) in the early follicular (EF), ovulatory (OV) and mid-luteal (ML) phase and oral contraceptive users at first (PC1) and second (PC2) pill consumption time points and during the pill free interval (PFI). *Indicates a significant post-hoc difference between phases (P < 0.05).
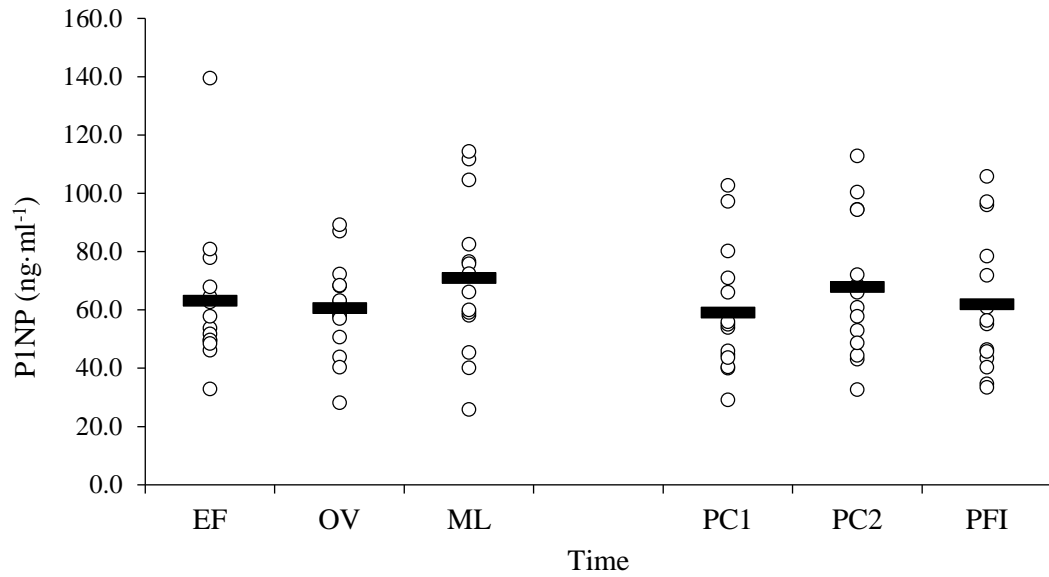
Figure 8.3. Univariate scatter plots with individual data points and mean values for Procollagen type 1 N telopeptide (P1NP) in the early follicular (EF), ovulatory (OV) and mid-luteal (ML) phase and oral contraceptive users at first (PC1) and second (PC2) pill consumption time points and during the pill free interval (PFI).
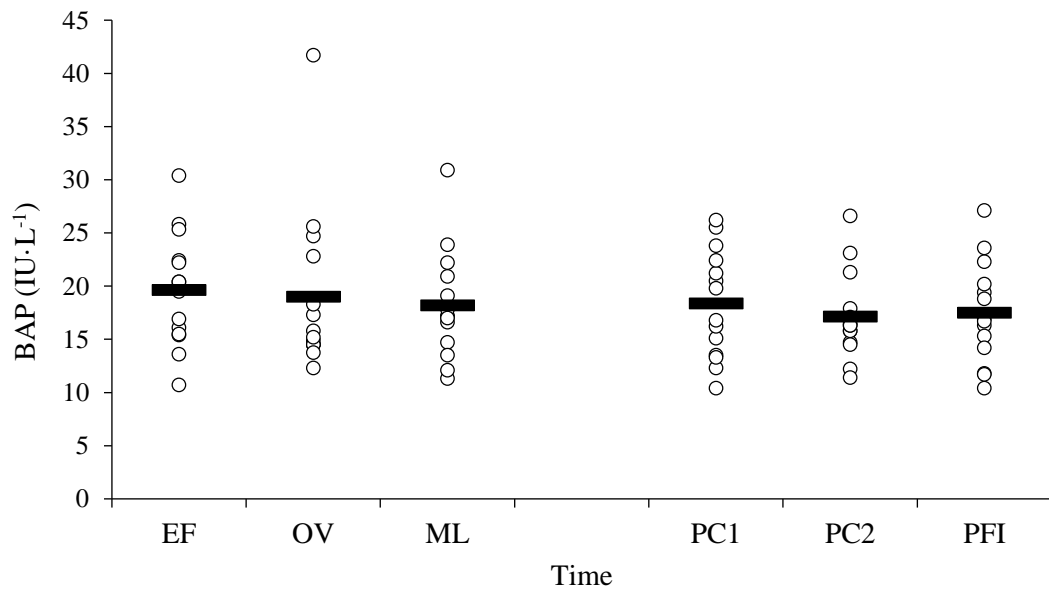
Figure 8.4. Univariate scatter plots with individual data points and mean values for bone-specific alkaline phosphatase (BAP) in the early follicular (EF), ovulatory (OV) and mid-luteal (ML) phase and oral contraceptive users at first (PC1) and second (PC2) pill consumption time points and during the pill free interval (PFI).

Table 8.1. Percentage differences in bone marker concentrations between phases of the menstrual cycle and oral contraceptive cycle. *Indicates a significant post-hoc difference between phases (P < 0.05). N.B. the reference phase for the percentage difference calculation is the second-mentioned phase *e.g.,* where 'EF vs. OV' is 5.9%, this states that mean EF values are 5.9% higher than those in OV.

	β-CTX	P1NP	BAP
Eumenorrheic			
EF vs. OV	+5.9%	+4.2%	+3.3%
EF vs. ML	+6.7%	-11.0%	+8.0%
OV vs. ML	-0.4%	-14.6%	+4.5%
Oral contraceptive			
PC1 vs. PC2	+16.0%*	-12.9%	+7.3%
PC1 vs. PFI	+1.2%	-4.6%	+5.0%
PC2 vs. PFI	+12.8%	+9.3%	-2.1%

Bone-specific alkaline phosphatase, BAP; Carboxy-terminal cross-linking telopeptide of type 1 collagen, β-CTX; Early follicular, EF; Mid-luteal, ML; Ovulatory, OV; Pill consumption, PC; Procollagen type 1 N telopeptide, P1NP.

8.3.3. Bone marker correlations

For eumenorrheic participants, ML phase oestradiol concentrations were significantly negatively correlated with EF phase BAP concentrations ($P = 0.007$, $r = -0.681$), with no other significant correlations being shown with oestradiol. EF phase β-CTX concentrations were positively correlated to OV phase and ML phase P1NP concentrations ($P < 0.05$; $r = 0.798$-0.838). β-CTX and P1NP were correlated during the OV phase ($P = 0.017$; $r = 0.626$), and ML phase β-CTX concentrations were correlated to P1NP at all time points ($P < 0.05$; $r = 0.662$-0.926).

For OC users, PC2 oestradiol concentrations were significantly negatively correlated to PFI β-CTX concentrations ($P = 0.041$, $r = -0.550$), with no other significant correlations to oestradiol. BAP concentrations at PC2 were significantly positively correlated to P1NP concentrations at PC1 ($P = 0.001$, $r = 0.764$) and PFI ($P = 0.005$, $r = 0.700$). β-CTX and P1NP concentrations were positively correlated at all time points ($P > 0.05$; $r = 0.638$-0.841).

8.4. Discussion

There were no significant differences in bone metabolism between eumenorrheic participants and OC users. Bone metabolic marker concentrations were also not different between menstrual cycle phases and, while β-CTX and BAP were not different between OC phases, P1NP was significantly (+16%) higher during late pill consumption compared to early pill consumption. Oestradiol was only correlated to BAP in eumenorrheic participants and β-CTX in OC users, although these correlations occurred with oestradiol concentrations from the preceding phase, evidencing a time lag of approximately 8 days in both instances.

In eumenorrheic participants, mean β-CTX concentrations were 6.3% and 6.7% lower in the ovulatory and mid-luteal phases compared to the early follicular phase, although this was not significant. For both the ovulatory and mid-luteal phases, 8 out of 14 participants β-CTX concentrations were reduced compared to the early follicular phase, with a wide range of individual responses (+35.0% to -60.2%), showing that this was a non-uniform effect. This contrasts with previous studies where β-CTX concentrations were significantly (~9-14%) lower in the follicular phase compared to the luteal phase (Gass et al., 2008; Liakou et al., 2016; Mozzanega et al., 2013; Niethammer et al., 2015). Individual variations in β-CTX concentrations have either been unreported in previous menstrual cycle research (Gass et al., 2008) or were relatively high; with standard deviations being 36-55% (Mozzanega et al., 2013) and 59-60% (Niethammer et al., 2015) of total β-CTX concentrations, similar to the current study (31-36%). Furthermore, the variability in responses between phases was large, with standard deviation of the total change ~30% of total values (Niethammer et al., 2015) and standard deviations of the % change greater than the actual % change (Gass et al., 2008). Large standard deviations and inter-individual responses reduce the likelihood of significant differences occurring as these are integral to the calculation of the *t* statistic. One reason why significant differences may have been observed in previous research is due to less stringent statistical procedures being employed such as non-corrected multiple comparisons (Gass et al., 2008) or more flexible α corrections for repeated comparisons (Tippets step-down procedure; Mozzanega et al., 2013), which significantly increase the likelihood of type 1 errors in these studies. This discrepancy in statistical approaches may also be responsible for the differences in P1NP results between the current study and previous research. P1NP concentrations were not significantly different across the menstrual cycle despite mean values being 14.6% higher in the ML phase compared to the OV phase. The absolute difference was greater than the 6.4% significant difference previously shown by Gass et al. (2008). The current study highlights that the changes between menstrual cycle phases for P1NP and β-CTX concentrations are not as clear as previous research suggests, and that large individual

variations in bone marker concentrations, coupled with individuality of responses between different phases, affects the interpretation of results.

In OC users, β-CTX concentrations on D15-16 pill consumption were significantly lower than D2-3 pill consumption (16.0%) and the PFI (14.6%), although this was not significant. Reduced β-CTX concentrations after approximately two weeks pill consumption is similar to previous research (Zittermann et al., 2002), however Zitterman et al. (2002) also showed reduced concentrations in the first week (day 3-5) of pill consumption, which was not observed in the current study. This disparity may be due to an earlier sampling date during pill consumption in the current study (day 2-3), where the effects of synthetic hormones may not yet have manifested, or may be due to analytical differences whereby Zitterman et al. (2002) used urinary β-CTX, which may be influenced by changes in creatinine excretion across the OC cycle (Brändle et al., 1992), while the current study measured β-CTX in serum which avoids this potential measurement error. Typically, low oestradiol concentrations are associated with an increased rate of bone resorption (Krassas & Papadopoulou, 2001), although the lowest β-CTX concentrations occurred on D15-16 pill withdrawal at a time where endogenous oestradiol concentrations were lowest. As circulating EO concentrations are elevated by > 50% during late pill consumption and activate ERs in a similar manner to endogenous oestrogen (Rabe et al., 2000), this may suggest that differences shown across the pill cycle were due to an inhibitory effect of synthetic oestrogens on bone resorption. Alternatively, this may be due to delayed effects of endogenous oestradiol as β-CTX concentrations during the PFI were negatively correlated with oestradiol measured 8-9 days earlier on D15-16 pill consumption. This is in line with other studies showing that the effect of oestradiol may occur with a time-lag, as these processes are based upon protein transcription activities that can take approximately one week to occur (Chiu et al., 1999; Gorai et al., 2014). The relative role of endogenous and exogenous reproductive hormones across the OC cycle is still unclear and requires further research to attribute any potential changes to synthetic or naturally-occurring hormones, although this study has shown that bone resorption does change across an OC cycle.

Oral contraceptive phase did not significantly affect P1NP concentrations, although mean P1NP concentrations were 12.9% higher on D15-16 pill consumption compared to D2-3, with 11/14 participant's P1NP concentrations increasing and changes ranging from -8.1% to +70.8%. As with other metabolic markers, the lack of significant difference may be due to high inter-individual variation (36-39%) and the large variation in the response between phases. P1NP has only been studied across an OC cycle on one other occasion, where there was a 21% reduction in P1NP concentrations between the PFI and day 18-21 pill consumption

(Wreje et al., 2000). In this study (Wreje et al., 2000), no statistical analysis was conducted, data were collected in a clinical population with chronic posterior pain that may have affected results (Kristiansson et al., 1996), and samples were collected after only two months OC use, which may affect the bone metabolic response (Endrikat et al., 2004b). This is the first study to assess P1NP across an OC cycle in a healthy population and has shown that there was no significant difference in P1NP concentrations between phases.

BAP concentrations did not vary across the menstrual cycle or between pill consumption phases. This is the first study to examine BAP across an OC cycle; the lack of change in BAP between menstrual cycle phases is similar to the majority of previous research (Gass et al., 2008; Niethammer et al., 2015; Shimizu et al., 2009). BAP is a marker of enzymatic activity during late stage mineralisation of bone, and therefore the lack of change between menstrual cycle and OC phases is somewhat expected as this mineral deposition may occur over protracted periods.

Despite significantly different reproductive hormone profiles, with eumenorrheic participants displaying significantly higher oestrogen concentrations compared to OC users, both of which had been consistently high/low for a minimum of 6 months, there were no differences in β-CTX, P1NP or BAP concentrations between groups. Many other studies have demonstrated that OC use results in reduced bone marker concentrations (Garnero et al., 1995; Glover et al., 2009; Karlsson et al., 1992; Ott et al., 2001; Paoletti et al., 2004; Rome et al., 2004; Wreje et al., 2000), although this is not unequivocal (Endrikat et al., 2004; Gargano et al., 2008; Nappi et al., 2003; Nappi et al., 2005), and the current study showed no differences. The between-group comparisons in the current study were conducted using mean values from three different phases of the menstrual cycle and OC cycle, and therefore may be more representative of bone metabolic marker concentrations compared previous research, that has used measurements from one time point.

This study has demonstrated that BAP and P1NP concentrations were not changed between different phases of the menstrual or OC cycles and β-CTX concentrations were not different between phases of the menstrual cycle, although they were significantly affected by pill consumption phase. This may have implications for the clinical use of bone metabolic markers, where contraceptive use is only considered as an uncontrollable source of pre-analytical variability in the long term (e.g. use or non-use; Vasikaran et al., 2011), although this study has shown that the phase within the OC cycle affects bone resorption. This study has improved upon previous research by controlling for exercise, fasting status, time of day and used a homogenous OC group, while employing more stringent statistical analyses to avoid type 1

errors. This research shows that synthetic reproductive hormones can affect bone metabolism independent of changes in energy availability and, therefore, the use of HCs, should be considered when examining future research in the Triad and RED-S models.

Chapter 9.0. The effect of low energy availability achieved through diet or exercise on muscle force in oral contraceptive users and non-users

9.1. Introduction

In Chapter 6, it was shown that reproductive hormones do not influence muscle force when energy availability is not manipulated. It is currently unclear, however, whether the reproductive hormone environment affects the response to low energy availability for muscle strength, in a similar manner to bone metabolism in the triad (De Souza et al., 2014; Mountjoy et al., 2014). Low energy availability has been demonstrated to negatively affect muscle protein synthesis (Areta et al., 2013) and impair sporting performance (Vanheest et al., 2014), although few studies have assessed the short-term effects of low energy availability on muscle strength.

Previous studies assessing the effects of short-term weight loss on muscle strength in weight category athletes are confounded by the negative effects of dehydration on muscle function (Artioli et al., 2010; Savoie, Kenefick, Ely, Cheuvront, & Goulet, 2015), while studies of malnourished or obese patients have used severely restrictive diets, which are not representative of athlete populations (Lopes, Russell, Whitwell, & Jeejeebhoy, 1982; Russell, Leiter, Whitwell, Marliss, & Jeejeebhoy, 1983). To date, only two studies have assessed the effects of short-term (2 weeks) energy restriction on muscle strength. Zachwieja et al. (2001) studied the effects of two weeks of moderate dietary restriction in males (n = 13) and females (n = 11), where daily energy intake was reduced by 750 kcal in the diet group (n = 16) and maintained in the control group (n = 8), with both groups undertaking 500 kcal of treadmill exercise each day. There was no change in 1 repetition maximum performance for leg press or shoulder press from pre- to post-condition in either group, suggesting minimal effects of energy restriction on strength in this population. Parkes et al., (1998) studied the effects of short-term, controlled dietary restriction on muscle strength in an active female population. Participants exercised for an average of 99 minutes per day and received either 100% (n=7) or 75% (n=7) of their energy requirements over 14 days. Isometric torque of the knee extensors was measured at baseline and post-intervention and produced inconsistent results; a reduction in concentric peak torque and an increase in eccentric average torque was observed for the 75 % group, whereas the 100% control group improved indices of concentric and eccentric average torque. The variable response in the control group suggests that these measures may not have been an accurate reflection of muscle function, possibly due to inadequate standardisation of positioning during assessments as indicated by the authors. Compounding this, a non-crossover design was used and the authors failed to control for menstrual cycle phase or OC use which may have influenced results (Phillips et al., 1996).

It is important to consider reproductive hormone status in studies assessing low energy availability as LH pulsatility is disrupted at an energy availability < 30 kcal·kgLBM^{-1}·day^{-1} resulting in a functional hypothalamic amenorrhea and a down-regulation of reproductive hormones (Loucks & Thuma, 2003). Low oestrogen concentrations have been associated with changes in metabolism (Bemben, Boileau, Bahr, Nelson, & Misner, 1992; Isacco, Duché, & Boisseau, 2012) and a greater degree of exercise-induced muscle damage (Joyce, Sabapathy, Bulmer, & Minahan, 2014; Minahan, Joyce, Bulmer, Cronin, & Sabapathy, 2015a). Therefore, reproductive hormone status may influence the metabolic response to low energy availability and may also mediate the subsequent effects on muscle function. Hormonal contraceptives are used by ~50% of athletes (see Chapter 5) and result in endogenous oestradiol concentrations analogous to hypothalamic amenorrhea and accordingly the responses to low energy availability should be studied in both HC users and eumenorrheic women to examine whether there are different responses.

Low energy availability is caused by a restriction of dietary energy intake, increased exercise energy expenditure, or a combination of both. Endurance athletes such as elite runners (Fudge et al., 2006) and professional cyclists (Vogt et al., 2005) exhibit an energy availability as low as 8 kcal·kgLBM^{-1}·day^{-1}, predominately due to increased energy expenditure in training. In contrast, other athletes such as figure skaters (Ziegler, Nelson, Barratt-Fornell, Fiveash, & Drewnowski, 2001) experience low energy availability primarily due to restricted dietary intake and low energy availability is seen in populations with disordered eating in the absence of exercise (Abraham, Pettigrew, Boyd, & Russell, 2006). It is therefore also necessary to assess if there are different responses to low energy availability when they are achieved through diet or exercise on muscle function.

Previous research examining the role of acute energy restriction has used; 1. unreliable measures of muscle function (Parkes et al., 1998), 2. muscles with anomalous properties in clinical patients (Lopes et al., 1982; Russell et al., 1983; Russell et al., 1983), 3. moderate energy restriction which is not representative of practices of those with low energy availability (Parkes et al., 1998; Zachwieja et al., 2001), 4. dehydration practices (Artioli et al., 2010). The aim of this study was to assess whether severe, short-term energy restriction affects muscle function and whether the effects are different when this is achieved through diet or exercise. Additionally, differences in responses between eumenorrheic and OC-using participants were examined.

9.2. Methods

9.2.1. Participants

Twenty recreationally active participants (10 eumenorrheic, 10 OC) who exercised for at least 4 h·week^{-1} as measured by the IPAQ (Craig et al., 2003), volunteered to take part in the study (Table 9.1). Eumenorrheic participants had a regular menstrual cycle in the 6 months prior to taking part with a cycle length of between 24-35 days, as confirmed with the menstrual cycle questionnaire (Appendix 12). Oral contraceptive participants used combined, monophasic low-dose formulations (Table 9.2) throughout the duration of the study and for at least 6 months prior to taking part, as confirmed with the OC details questionnaire (Appendix 13). All participants were not at risk of an eating disorder, as characterised by their score (≤ 2) on the SCOFF eating disorder questionnaire (Morgan et al., 1999). Participants were Caucasian, non-smoking and non-vegetarian due to the known effects on reproductive hormone concentrations and other metabolic factors (Herrmann et al., 2009; Marsh et al., 2011; Yoon, Maalouf, & Sakhaee, 2012). Exclusion criteria for participation were: aged < 18 or > 40 years, musculoskeletal injury, use of medication that may affect outcome measures, bone fracture in previous 12 months, history of metabolic, heart, liver or kidney disease, diabetes, thyroid disorders, breastfeeding women, women trying to become pregnant or women with amenorrhea, short, long, or irregular cycles. Participants were provided with an information sheet (Appendix 16), completed a health screen (Appendix 8) and gave their written informed consent (Appendix 17) to take part. The study was approved by the Nottingham Trent University Research (Human) Ethics Committee and the East Midlands NHS Research Ethics Committee (14/EM/1156).

Table 9.1. Participant characteristics for eumenorrheic and oral contraceptive participants.

	Eumenorrheic n = 10	Oral contraceptive n = 10
Age (y)	24.10 ± 2.92	25.89 ± 4.01
Height (m)	1.66 ± 0.06	1.65 ± 0.04
Body mass (kg)	61.06 ± 7.05	58.13 ±4.71
Physical Activity Level (METs)	4833.4 ± 2431.8	3385.6 ± 1196.1
SCOFF eating disorder score	0.50 ± 0.71	0.30 ± 0.71
Lean Body Mass (kg)	41.29 ± 4.10	41.11 ± 3.26
$\dot{V}O_2$max (ml·kg·min^{-1})	47.71 ± 3.75	49.61 ± 6.82

Maximal oxygen uptake, $\dot{V}O_2$max; Metabolic equivalent, METs

Table 9.2. Oral contraceptive formulation details.

Oral contraceptive brand	No. of participants using brand	Ethinyl oestradiol concentration	Progestin component	Progestin concentration
Microgynon®	5	30 µg	Levonorgestrel	150 µg
Yasmin®	2	30 µg	Drospirenone	3 mg
Rigevidon®	1	30 µg	Levonorgestrel	150 µg
Gederal®	1	30 µg	Desogestrel	150 µg
Milinette®	1	20 µg	Gestodene	75 µg

9.2.2. Experimental design

Prior to taking part in the experimental conditions, participants underwent an initial assessment and were familiarised with the muscle function assessment procedures. All participants then completed three, 3-day experimental conditions in a crossover design, randomised using a Latin-square allocation; controlled energy balance (BAL), diet-induced energy restriction (DIET) and exercise-induced energy restriction (EX). Dietary energy intake (DEI), exercise energy expenditure (EEE) and the resultant energy availability (EA) for each condition are displayed in Figure 9.1. For each condition, a blood sample and muscle function assessment was conducted at baseline (PRE) and in the morning immediately following (POST) the 3-day prescribed diet and exercise regimen. Within-participant testing was conducted at the same time for each session, with laboratory visits starting between 07:15 - 08:30. Participants were asked to consume ~500ml of water upon awakening and refrain from exercise in the 24h before PRE-testing. Each condition was completed in the EF phase of the menstrual cycle for eumenorrheic participants and the first week of pill consumption for OC users. Oral contraceptive users were asked to consume their pill 1 h prior to arrival to the laboratory and asked to consume it at this time for the duration of the study.
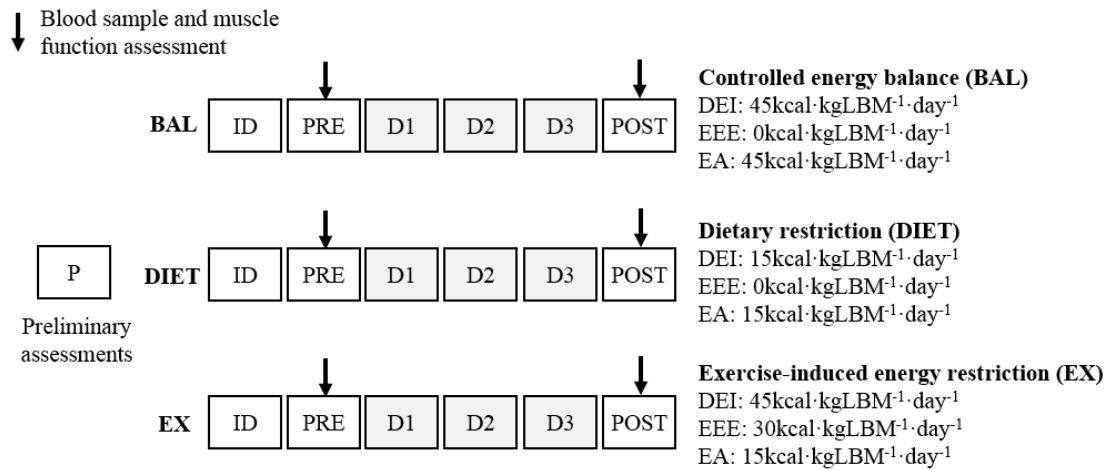
Figure 9.1. Overview of the study design. Preliminary assessments (P) were conducted, followed by the controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy restriction (EX) conditions. Identification (ID) of the first day of the menstrual cycle or pill consumption was followed by baseline testing (PRE), then the three-day diet and exercise regimen (D1-3) and follow-up testing (POST).

9.2.3. Experimental protocol

9.2.3.1 Preliminary assessment

Participants completed the IPAQ (Section 3.7.3) to assess physical activity levels and the SCOFF questionnaire (Section 3.7.4) to assess eating disorder risk. Lean body mass was measured using a DXA scan (Section 3.3.3) and an incremental treadmill test (Section 3.5) was used to measure $\dot{V}O_2max$.

9.2.3.2. PRE and POST condition testing

Upon arrival to the laboratory, height and body mass were measured (Section 3.3.1), 30 ml of blood was drawn from the medial antecubital vein and blood samples were analysed for oestradiol and T_3 as described in Section 3.8. Bone metabolic markers (P1NP and β-CTX) were also measured and results from EU participants are presented in Papageorgiou et al. (2018). These results are not discussed in the current thesis as this was a collaborative project with another doctoral student (MP) and these results formed part of her thesis. Oestradiol was measured to assess the differences in reproductive hormone profiles between groups and T_3 was measured to assess the effects of the different experimental protocols on energy metabolism and identify any between group differences as OC use influences T_3 production (Wiegratz et al., 2003). Following the blood sample, there was a ~30-minute period where participants remained sedentary and completed two short questionnaires and a battery of

cognitive tasks to assess cognitive function related outcomes that are discussed in Chapter 10. An assessment of quadriceps muscle force (Section 3.4.2) and FDI muscle force (Section 3.4.3) was then conducted using the standardisation procedures described previously (Section 3.4.1).

9.2.3.3. Diet and Exercise protocol

During the 3-day experimental period, participants were provided with diets matched to their caloric requirements for the experimental condition (Figure 9.1). Diets were individually weighed and separated into containers for participants. The chosen diets consisted of cereals, milk, vegetable soups, pitta bread, salad and meat/fish and were palatable and easy to prepare to enhance compliance. The macronutrient composition of the diets were 50% carbohydrate, 30% fat and 20% protein. During each 3-day experimental period, participants were asked to consume all the food provided and not to consume any other food or caloric beverages. Participants were instructed to consume the meals at similar times within and between conditions to limit the effects of different within-day energy deficiencies on responses to alterations to energy availability (Fahrenholtz et al. 2018), although it is acknowledged that within-day energy deficiencies were not calculated. During the DIET condition, a multivitamin and mineral supplement (Boots A-Z, UK) was provided for daily consumption. Participants were asked not to participate in any exercise during the experimental conditions (*e.g.,* cycling to work, running, gym, training), unless as part of the experimental protocol, however they could perform normal daily activities.

For the EX condition, participants performed treadmill running equivalent to 30 kcal·kgLBM·day^{-1}, which was separated into two exercise sessions, one in the morning and one in the afternoon. This consisted of repeated 15-minute exercise bouts at 70% $\dot{V}O_{max}$, with 5 minutes rest between bouts, until energy expenditure reached the required amount as determined through breath-by-breath analysis (ZAN600 CPET, nspire, USA). Breath-by-breath analysis was used for the initial morning exercise session and the values were used to calculate running speed and duration for all subsequent exercise sessions. Treadmill running was used as participants were accustomed to running and running has a greater effective load stimulus (4.88) compared to cycling (0.14; Weeks & Beck, 2008), thereby providing a greater osteogenic stimulus to assess the effects of exercise on bone metabolic responses, which were explored in a separate aspect to this study (Papageorgiou et al., 2018).

9.2.4. Statistical analysis

Independent samples t-tests were conducted to explore differences between EU and OC participants for baseline characteristics. Muscle force, body mass and biochemical data were assessed for normality and sphericity and analysed using a 3-way mixed-model ANOVA

(group x condition x time). Where sphericity was not assumed, Greenhouse-Geisser adjustments were made. Bonferroni adjusted post-hocs were used to assess significant differences. Effect sizes were calculated using Cohen's d (Cohen & Jacob, 1992) and were described as trivial (0.0 – 0.19), small (0.20 – 0.49), medium (0.50 – 0.79) and large (> 0.80). Data are represented as mean ± 1SD and statistical significance was set at $P \leq 0.05$.

9.3. Results

9.3.1. Participant characteristics

There were no differences between eumenorrheic and OC participants for age, height, weight, $\dot{V}O_2max$, LBM, baseline physical activity levels and eating disorder risk (all $P > 0.05$).

9.3.2. Oestradiol

Mean oestradiol concentrations were significantly higher in eumenorrheic (142.1 ± 62.6 pmol·L^{-1}) than OC (51.2 ± 53.5 pmol·L^{-1}) participants ($P < 0.001$; d = 1.24). There was a significant time x group interaction ($P = 0.001$) with oestradiol concentrations reduced from pre (73.8 ± 68.1 pmol·L^{-1}) to post (28.6 ± 11.2 pmol·L^{-1}; $P = 0.002$; d = 1.10) condition in OC participants and increased from pre (125.4 ± 59.9 pmol·L^{-1}) to post (160.8 ± 61.1 pmol·L^{-1}) in eumenorrheic participants ($P < 0.001$; d = 0.51; Table 3). There was no effect of condition on oestradiol concentrations and condition did not interact with any other level (all $P > 0.05$).

Table 9.3. Oestradiol concentrations (pmol·L^{-1}) for eumenorrheic and oral contraceptive participants pre and post each condition for controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy restriction (EX). *Indicates a significant ($P < 0.05$) difference compares to PRE values.

Condition	Eumenorrheic		Oral contraceptive	
	PRE	POST	PRE	POST
BAL	108.9 ± 33.6	157.3 ± 53.1	68.9 ± 63.3	27.6 ± 9.8
DIET	118.9 ± 29.7	159.0 ± 62.9	71.9 ± 52.8	29.1 ± 11.4
EX	148.3 ± 92.9	167.0 ± 72.1	80.7 ± 89.8	29.0 ± 13.3
Mean	125.4 ± 49.9	160.8 ± 61.1*	73.8 ± 68.1	28.6 ± 11.2*

9.3.3. T_3 concentrations

Mean T_3 concentrations were significantly lower in eumenorrheic (1.45 ± 0.26 nmol·L^{-1}) compared to OC (1.78 ± 0.31 nmol·L^{-1}) participants ($P = 0.019$; d = 1.06), although the group did not influence the response over time or between conditions ($P > 0.05$). There was a main

effect of time (P = 0.037), with T_3 concentrations higher pre-condition (1.66 ± 0.34 nmol·L^{-1}) compared to post-condition (1.59 ± 0.38 nmol·L^{-1}; d = 0.19), and a main effect of condition (P = 0.008), with higher T_3 concentrations in BAL (1.67 ± 0.36 nmol·L^{-1}) compared to DIET (1.57 ± 0.35 nmol·$L^{-1;}$ d = 0.28). There was a significant condition x time interaction (P < 0.001), with a reduction in T_3 concentrations from pre to post condition in DIET (P < 0.01; d = 0.67), with no change in BAL or EX (P > 0.05; Figure 9.2).
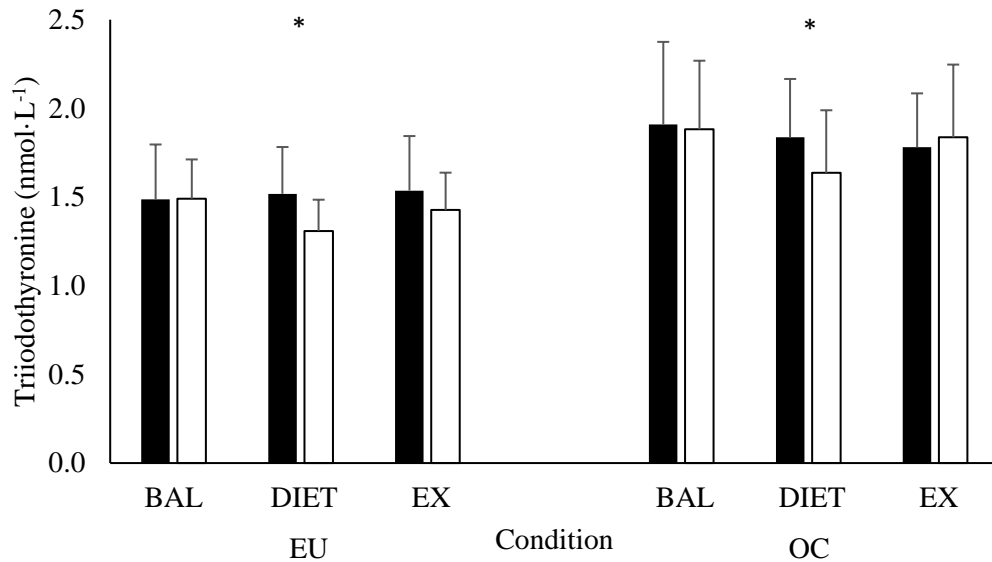
Figure 9.2. Triiodothyronine concentrations pre-condition (black bars) and post-condition (white bars) for eumenorrheic (EU) and oral contraceptive (OC) participants for controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy restriction (EX). *Indicates a significant condition x time reduction from pre to post condition (P < 0.05).

9.3.4. Body mass

There was a significant main effect of time (P < 0.001), with body mass reduced from pre (59.9 ± 5.9 kg) to post (58.8 ± 5.8 kg) condition (d = 0.19; Table 9.4). There was a significant condition x time interaction (P < 0.001), with DIET (-3.1 ± 1.1 %) resulting in a greater reduction in body mass than EX (-1.6 ± 1.0 %; P = 0.018; d = 1.43) and BAL (-1.0 ± 0.8 %; P < 0.001; d = 2.21) and EX also resulting in a greater reduction in body mass than BAL (P = 0.03; d = 0.67). There was no effect of group over time or between conditions (P > 0.05).

Table 9.4. Body mass (kg) pre-condition (PRE) and post-condition (POST) and % change for controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy

restriction (EX). *Indicates significantly different to BAL and † indicates significantly different to EX (P < 0.05).

Condition	PRE	POST	% CHANGE
BAL	59.8 ± 6.1	59.2 ± 6.0	-1.0 ± 0.8
DIET	60.1 ± 6.0	58.2 ± 5.9	-3.1 ± 1.1 *†
EX	59.8 ± 5.8	58.9 ± 5.6	-1.6 ± 1.0 *

9.3.5. Muscle force production measurements

Quadriceps muscle force was not different between groups (P = 0.238) and group did not interact with any other level (P < 0.05). There was a significant condition x time interaction (P = 0.007) where quadriceps force was unchanged from pre to post in BAL and DIET conditions (P > 0.05), with a 13.2% decline in quadriceps force from pre to post EX (P = 0.01; d = 0.53; Figure 9.3).

FDI muscle force was not different between groups (P = 0.966) or conditions (P = 0.576) and force was maintained from pre-to post in all conditions (P = 0.167) with no significant interaction effects at any point (P > 0.05; Figure 9.4).
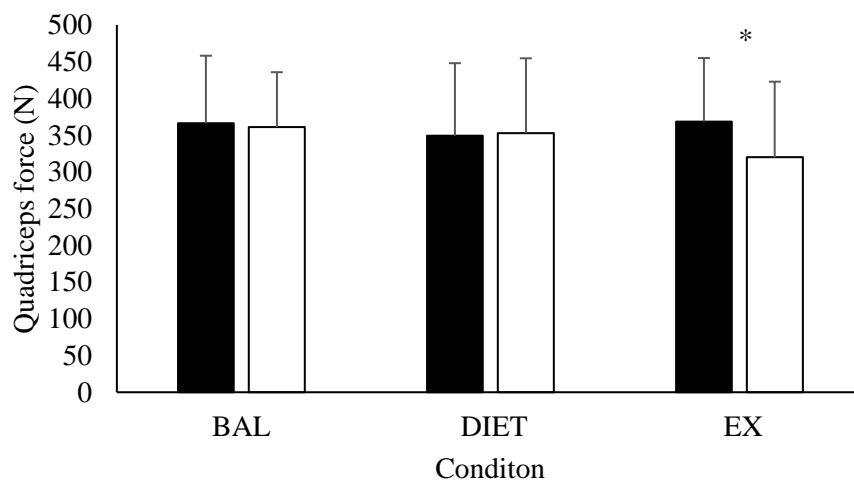
Figure 9.3. Quadriceps force pre-condition (black bars) and post-condition (white bars) for all participants for controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy restriction (EX). *Indicates a significant condition x time reduction from pre to post condition (P < 0.05).
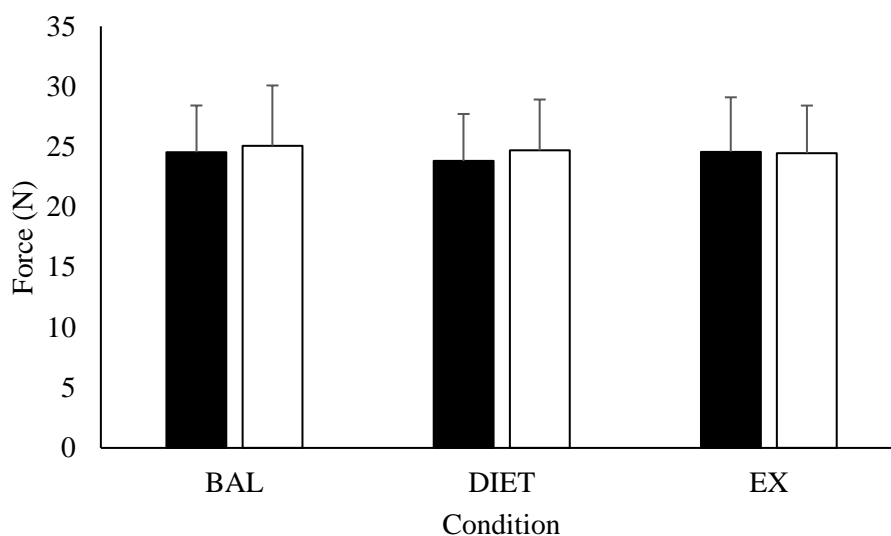
Figure 9.4. First dorsal interosseus (FDI) muscle force pre-condition (black bars) and post-condition (white bars) for all participants for controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy restriction (EX).

9.4. Discussion

The current study compared eumenorrheic women and OC users, the two most prevalent reproductive hormone models in female athletes (Chapter 5), to examine if there were differences in the muscle force response to low energy availability. Similar to Chapter 6, the reproductive hormone environment did not affect the muscle force response, indicating that reproductive hormones do not influence the short-term response to low energy availability for muscle strength. Low energy availability achieved through dietary restriction had no effect on quadriceps or FDI muscle force production, whereas low energy availability caused by exercise resulted in a significant decline (13.2%) in force production of the quadriceps, with no change in FDI muscle force.

Muscle force was measured in both the FDI muscle, which remained passive during the exercise protocol, and the quadriceps, which are heavily loaded during treadmill exercise. As a decline in muscle force was only evident in the quadriceps during the EX condition, with no change in FDI muscle force, it appears that the force impairment was a result of the exercise protocol directly on the exercised muscle, rather than the exercise or low energy availability affecting the muscular system in general. The probable cause of reduced quadriceps force following the EX condition was exercise-induced muscle damage, which typically occurs after prolonged, low force, eccentric exercise (Byrne, Twist, & Eston, 2004), as used in the current

study. This is characterised by a disruption of the sarcomeres and alterations to the excitation-contraction coupling system (Gissel & Clausen, 2001; Proske & Morgan, 2001; Warren, Ingalls, Lowe, & Armstrong, 2001), which can result in a loss of muscle force for several days post exercise (Byrne et al., 2004). The 13.2% decline in quadriceps force in the current study is similar to that shown following muscle damage protocols or prolonged periods of running such as marathons (Minahan et al., 2015a; Petersen et al., 2007).

Prolonged or severe exercise, such as in the current study, can alter the contractility and structural integrity of the muscle independent of mechanical damage (Belcastro et al., 1998; Waterman-Storer, 1991). Excessive exercise can result in an inability of the muscle to buffer changes in Ca^{2+}, with sustained Ca^{2+} elevations having been shown to trigger rapid cellular damage (Duncan, 1987). Increased Ca^{2+} concentrations stimulate calpains, which results in the immediate degradation of myofibrillar structural proteins including tropomyosin, troponin and titin (Huang & Forsberg, 1998). This causes a decentralisation of myosin filaments within the sarcomere, Z-line streaming, disturbances to the sarcoplasmic reticulum T-tubular system and a loss of sarcolemmal integrity (Belcastro et al., 1998; Huang & Forsberg, 1998; Murphy, 2010). Calpain activity has been shown to be elevated by 18-22% following intensive exercise and has an immediate effect on protein degradation (Belcastro et al., 1996). The putative role of calpain on muscle structural protein degradation during and post exercise may be especially important for athletes who exercise with low energy availability as calpain is also elevated as a result of dietary restriction (Belcastro et al., 1996). Therefore, it may be that the low energy availability exacerbated the calpain-based muscle structural protein breakdown as a result of exercise, and that the decline (-13.2%) in muscle force may not have been as severe with the same amount of exercise, but with a balanced level of energy availability.

The effect of exercise and energy restriction on muscle calpain and force production was studied by Parkes et al. (1998), who found that exercising for an average of 100 minutes per day with 75% of energy requirements for 14 days was not sufficient to alter muscle calpain activity or affect knee extensor torque. The difference in results between the current study and Parkes et al. (1998) may be due to differences in exercise duration (~130 min·day^{-1} vs. ~100 min·day^{-1}), mode (treadmill running vs. mixed-mode exercise), degree of energy restriction (~33% vs. 75% of dietary requirements) or because the participants in the current study were less accustomed to the exercise. Participants in Parkes et al. (1998) were asked to continue their habitual exercise practices, which involved several exercise modes (primarily running/swimming/cycling), with daily energy expenditure estimated based upon participant's records of exercise participation and REE measurements. In contrast, participants in the current study were prescribed an exercise protocol whereby the energy expenditure was

directly measured, with an exercise duration that was typically greater than participant's habitual exercise levels. Another reason for the different responses may be that the current study used isometric force measurements, which have a lower variability and are more reliable (Gleeson & Mercer, 1996), so there is less chance for spurious results as seen in Parkes et al. (1998) where there was a variable response in the control group. In the current study, both quadriceps and FDI muscle force were unchanged from pre to post in the BAL condition.

The unchanged muscle force production after dietary restriction in both the quadriceps and FDI muscle is in contrast to previous research in weight category sports where dietary restriction was associated with reductions in muscle strength (for review see Artioli et al., 2010). However, the rapid weight loss employed in these sports was typically concurrent with dehydration strategies in order to meet specific weight targets, which has been shown to negatively influence muscle strength (Savoie et al., 2015). Unlike applied rapid weight loss research, participants in the current study were allowed to consume water *ad libitum* and were not instructed to reach specific weight targets, so it is unlikely that participants were severely dehydrated. Severe dietary restriction (~400 kcal·day^{-1}) has previously been shown to alter muscle force-frequency characteristics (Lopes et al., 1982; Russell et al., 1983), which lead to muscle function assessments of the adductor pollicis being used to identify malnutrition prior to other clinical symptoms in anorexia nervosa patients (Jeejeebhoy, 1986). This is the first study to show that, using a controlled dietary intake, severe short-term dietary restriction does not influence force production of important locomotor muscles such as the quadriceps.

The muscle force response to low energy availability through diet and exercise was similar between eumenorrheic women and OC users, with both groups only exhibiting a decline in force from pre to post EX condition. This is despite differences in reproductive hormone profiles between groups, with eumenorrheic women displaying higher endogenous oestrogen concentrations compared to OC users and differences within groups; eumenorrheic participants oestrogen concentrations increased from pre- to post-condition during the early follicular phase and OC users oestrogen concentrations were reduced from pre- to post-condition in the first week of pill consumption (Stricker et al., 2006; Willis et al., 2006). Independent of changes in energy availability, our group has previously shown that muscle force is not influenced by reproductive hormone concentrations (Elliott, Cable, Reilly, & Diver, 2003; Elliott et al., 2005; Chapter 6). However, oestrogen and ERα expression have been demonstrated to attenuate post-exercise calpain concentrations (Gamerdinger, Manthey, & Behl, 2006; Tiidus et al., 2001) and is associated with antioxidant and membrane stabilization properties thought to limit the occurrence of exercise-induced muscle damage (Komulainen et al., 1999; Tiidus, 2003). Previously, it has been shown that OC users exhibit

a greater degree of exercise-induced muscle damage (Joyce et al., 2014; Minahan, Joyce, Bulmer, Cronin, & Sabapathy, 2015b) and a delayed strength recovery (Savage & Clarkson, 2002) compared to eumenorrheic women. Nevertheless, despite significant differences in reproductive hormone concentrations between groups, there was no difference in the degree of muscular force impairments between OC users and eumenorrheic participants in the current study. The lack of differences between groups may be explained by the timing of the experimental conditions; the current study was conducted during first week of pill consumption for OC users where exogenous oestrogen was supplied to participants, whereas in previous research, between group differences have been observed in the PFI (Joyce et al., 2014; Minahan et al., 2015b; Savage & Clarkson, 2002). We chose the pill consumption phase as this is more representative of OC user's habitual status, with the majority of pill regimens consisting of 21 pill consumption days and 7 PFI days. If the study was repeated with the experimental periods taking place during the withdrawal phase, the omission of exogenously supplied oestrogen may lead to a greater decline in muscle function in the OC group compared to the eumenorrheic group. It must also be taken into consideration that the eumenorrheic group were tested during the early follicular phase to limit the differences in oestrogen concentrations pre and post experimental conditions, however exercise-induced muscle damage has been shown to be reduced in the luteal phase when oestrogen concentrations are highest (Williams, Walz, Lane, Pebole, & Hackney, 2015). Future research should examine if the timing of experimental conditions affects between group differences in the force response to muscle-damaging exercise.

As expected, there were also differences in T_3 concentrations between groups, with OC users having greater T_3 concentrations overall (Wiegratz et al., 2003). T_3 is an important regulator of energy metabolism and its production is down-regulated in response to low energy availability in an adaptive response to conserve energy (McAninch & Bianco, 2014). T_3 has previously been used to assess the energy conservation response to periods of low energy availability (De Souza et al., 2008; Vanheest et al., 2014), however it was not known whether the change in T_3 in response to low energy availability would be different between OC users and non-users. Both groups responded similarly, showing a decline in T_3 concentrations from pre to post DIET, which was not observed in BAL or EX conditions. In rodents, T_3 production has been demonstrated to be elevated in response to exercise (Katzeff, Bovbjerg, & Mark, 1988) and maintained at normal levels when exercising to induce an energy deficit (Katzeff & Selgrad, 1991). Therefore, the different T_3 response between DIET and EX conditions is not indicative of different levels of energy availability. In fact, this highlights that T_3 may not be a suitable measure to determine if athletes have an energy conservation response to low energy availability as it is mediated by exercise.

The change in body mass in response to the different experimental conditions was similar between eumenorrheic women and OC users, but varied between conditions. Whilst both the DIET and EX conditions resulted in a greater loss of body mass than BAL over the 3-day experimental period, there was a greater reduction in body mass in the DIET condition compared to EX. This was likely due to muscle glycogen being prioritised as a fuel source during dietary restriction (Krause & Mahan, 1980), with each gram of muscle glycogen stored with 3-4 g of water (Olsson & Saltin, 1970). In contrast, the exercise may have promoted post-exercise glycogen synthesis (Ryan et al. 2012) so that glycogen stores were replenished, contributing to a lower body mass loss over the 3 days compared to DIET. An exercise stimulus results in the synthesis of muscle proteins, which retains body water and also results in a conservation of protein stores and an enhanced oxidation of fat as an energy source (McMurray, Ben-Ezra, Forsythe, & Smith, 1985), which is half as calorically dense as protein or carbohydrate. Therefore, the differences in body mass changes between DIET and EX conditions are explainable, have been previously observed (McMurray et al., 1985) and is not indicative of differences in energy availability between the conditions.

Short-term dietary restriction had no effect on muscle force production in either the quadriceps or FDI muscle. In contrast, when low energy availability was achieved by increased exercise energy expenditure, force was reduced in the quadriceps, which were heavily loaded during exercise, with no change in the FDI muscle, which remained passive during exercise. The decline in quadriceps muscle force was most likely due to exercise-induced muscle damage, however it is unclear if the same level of force decrement would have occurred if the exercise protocol was completed when energy replete. There were no differences in force production response between eumenorrheic participants and OC users, however future research should explore the timing of experimental protocols in relation to the pill consumption day and menstrual cycle. These findings highlight that athletes that have a low energy availability due to engaging in large volumes of exercise may have reduced muscle strength in the exercised muscle, which could affect exercise performance or injury risk. This study has added to the evidence from Chapter 6, that the reproductive hormone environment is not an important moderating factor in relation to muscle force production, in both low energy availability and energy replete populations.

Chapter 10.0. The effect of low energy availability achieved through diet or exercise on cognitive function in oral contraceptive users and non-users

10.1. Introduction

In the previous chapter it was shown that muscle strength, one of the proposed performance factors affected by RED-S (Mountjoy et al., 2014), was not affected by low energy availability, unless the low energy availability was achieved by exercise, where only the exercised muscle was affected. In addition to muscle strength, the RED-S model also states that aspects of cognitive function such as judgement, concentration and co-ordination are affected by reduced energy availability, however there is currently little evidence available to support this (De Souza et al., 2014).

To date, only two studies have examined the effects of short term (2-3 days) low energy availability on cognitive performance. In the first study, two days of near total calorie deprivation (~183 kcal·day^{-1} energy intake), was compared to energy-balanced conditions, consisting of either carbohydrate or carbohydrate and fat diets (~2820 kcal·day^{-1} energy intake), with all conditions including two hours of low intensity exercise (40-45% heart rate reserve) per day (Lieberman et al., 2008). Consuming the calorie-restricted diet did not impact participant's self-reported mood and had no effect on vigilance, response time, memory, and reasoning skills. In the second study, participants completed approximately four hours of exercise per day (40-65% $\dot{V}O_2$peak) and consumed either an energy-balanced (3935 ± 769 kcal·day^{-1} energy intake), or calorie-restricted (266 ± 61 kcal·day^{-1} energy intake) diet (Lieberman et al., 2017). Mood was significantly affected, with reduced vigour and increased tension, fatigue and total mood disturbance in the calorie-restricted condition, which was associated with a significant reduction in interstitial glucose concentrations. Performance on the majority of cognitive tests was unaffected, however aspects of grammatical reasoning and choice reaction time were improved in the calorie-restricted condition, compared to the energy-replete condition.

In these studies (Lieberman et al., 2008, 2017), changes in cognitive performance were only apparent when a greater exercise duration and intensity was used (Lieberman et al., 2017), which resulted in a greater deviation from energy balance (-3681 kcal·day^{-1} compared to -2138 kcal·day^{-1} in the earlier study), despite similar dietary energy intakes. The severity of the dietary restriction combined with the exercise regimen in these studies would have resulted in a negative energy availability, which is only representative of extreme situations, such as military training, and do not represent practices of athletes. Energy availability in athletes is often between ~8-35 kcal·kgLBM^{-1}·day^{-1} (Doyle-Lucas, Akers, & Davy, 2010b; Vanheest et al., 2014; Viner et al., 2015; Vogt et al., 2005), which is achieved through more moderate levels of dietary energy restriction than previous cognitive research (Lieberman et al., 2008,

2017), or a failure to increase energy intake to compensate for exercise training (De Souza et al., 2014). Therefore, further research using more ecologically valid levels of energy availability and methods of achieving this (i.e. dietary restriction and/or exercise) is required. Furthermore, previous research (Lieberman et al., 2008, 2017) has not used a non-exercise control, or dietary restriction only group, which may affect results as exercise is known to affect cognitive function (Chang et al., 2012; Tomporowski, 2003).

Lieberman et al. (2017) identified an effect of sex on the response to calorie-restriction, whereby females (n = 6) performed better in tests of working memory, grammatical reasoning and vigilance in the calorie-restricted condition, which was not apparent in males (n = 17). In women, reproductive function is sacrificed during periods of low energy availability resulting in a down-regulation of reproductive hormones (Loucks et al., 1998), which may exacerbate the effects of calorie restriction on cognition, as oestrogen has been linked to changes in several domains of cognitive function (Luine, 2014). Further research is required to assess these effects in females, in addition to studying different models of reproductive functioning that may influence the response to energy restriction; combined OC users consume synthetic oestrogens and progestins and have down-regulated endogenous reproductive hormone concentrations, similar to those seen in amenorrheic women (Elliott-Sale et al., 2013), whilst oestrogen and progesterone concentrations are greater in eumenorrheic women and fluctuate in a cyclical manner across the menstrual cycle (Stricker et al., 2006).

Therefore, the aim of this study was to assess the effects of low energy availability, achieved through diet or exercise, on cognitive function in females, using an energy availability representative of athletic populations. A further aim of this study was to explore differences in responses between eumenorrheic women and OC users.

10.2. Method

10.2.1. Participants

The participant population was described Section 9.2.1 and all participants were English speakers.

10.2.2. Experimental Design

The experimental design was described in Section 9.2.2. Participants were randomly allocated to complete the experimental conditions in a counter-balanced order, using a Latin-square design, however, due to participant availability, 6 out of 20 participants were unable to complete the sessions in the specified order.

10.2.3. Experimental Protocol

10.2.3.1. Preliminary assessment

The preliminary assessment was described in Section 9.2.2. Participants were familiarised with the cognitive function battery prior to the main experimental trials.

10.2.3.2. PRE and POST condition testing

Upon arrival to the laboratory, height and body mass (Section 3.3.1) were measured and 30 ml of blood was drawn using venepuncture for analysis of oestradiol concentrations (Section 3.8). Bone metabolic markers and other hormones were also analysed and these data are presented in a separate doctoral thesis and Papageorgiou et al. (2018). Following this, participants completed the BRUMS questionnaire (Section 3.7.1) to assess mood state and the PSQI (Section 3.7.2) to measure self-reported sleep quality. Participants then completed the following test battery using the standardisation procedures described in Section 3.6.1:

- RAVLT (Section 3.6.3)
- Mental rotation test (Section 3.6.3)
- Visual Search test (Section 3.6.5)
- Stroop test (Section 3.6.6)
- RVIP (Section 3.6.7)

Verbal fluency performance was not measured as test equivalence has not been demonstrated for six alternate test versions as required in the current study due to the number of experimental trials.

10.2.3.3. Diet and Exercise protocol

The diet and exercise protocol during the experimental conditions was described in Section 9.2.2.3.

10.2.4. Statistical Analysis

A one-way repeated-measures ANOVA was used to assess for differences between conditions at baseline. Change from pre- to post-condition for cognitive function, BRUMS and PSQI data were calculated and analysed using a two-way (group x condition) (RAVLT, RVIP, BRUMS, PSQI) and three-way (group x condition x level) mixed-model ANOVA (mental rotation test, Stroop test, visual search test) using Statistica (Dell, USA). Oestradiol concentrations were analysed using a three-way (group x condition x time) mixed-model ANOVA. Data were checked for normality using Shapiro-Wilk tests and significant effects were explored with

Bonferroni-adjusted t-tests, with effect sizes calculated using Cohens' d (0.2 = small, 0.5 = medium, 0.8 = large; Cohen & Jacob, 1992). All data are presented as mean ± 1SD and statistical significance was set at $P \leq 0.05$.

10.3. Results

10.3.1. Cognitive function

The mean data for all cognitive function tests can be found in Table 10.1. There were no differences between conditions at baseline for any of the cognitive function measures (all P > 0.05), therefore data were analysed and are presented as change from pre- to post-condition. Significance values for cognitive function measures are presented in Table 10.2. The change from pre- to post-condition for the RAVLT, visual search test, Stroop test and RVIP task was not affected by condition, group or test level (visual search test, Stroop test), and there were no significant interactions between these factors.

The change from pre-to post-condition was different between conditions for accuracy in the mental rotation test (main effect of condition, P = 0.045). As change data were analysed, this is indicative of a divergence in performance over time, between conditions. Post-hoc tests showed that whilst accuracy improved in the BAL condition, there was a decline in accuracy in the EX condition (BAL, +2.5%; EX, -1.4%; P = 0.042, d = 0.85). DIET (+1.3%) was not different to BAL or EX (P > 0.05; Figure 10.1). There was a significant difference between groups for response times in the mental rotation test (main effect group, P = 0.017), whereby response time was improved to a greater extent from pre-to post-condition in OC users (-13.7%) compared to eumenorrheic participants (-4.0%, P = 0.017; d = 0.67). There was a main effect of level for mental rotation test response time (P = 0.006) and accuracy (P < 0.001). Response time was improved to a greater extent from pre-to post-condition at 80° compared to 0° rotation (P = 0.005, d = 0.29). Accuracy was improved to a greater extent from pre-to post-condition at 80° compared to 0° (P < 0.001; d = 0.72) and 20° rotation (P < 0.001; d = 1.38) and at 40° compared to 20° (P = 0.031; d = 0.90).

Table 10.1. Cognitive function absolute values (mean ± 1SD) and percentage change from pre- to post-condition for controlled energy balance (BAL), diet-induced energy restriction (DIET) and exercise-induced energy restriction (EX).

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

Rey Auditory Verbal Learning Test, RAVLT; Rapid Visual Information Processing, RVIP. Response time (RT) data are presented in ms, accuracy data are presented as proportion of correct responses and change data are presented as percentage. *Indicates a main effect of condition, with EX different to BAL (P < 0.05).

Table 10.2. Significance values for cognitive function measures for group (eumenorrheic and oral contraceptive), condition (controlled energy balance, diet-induced energy restriction and exercise-induced energy restriction) and level (simple and complex) main effects and interaction effects.

	Main effect Group	Main effect Condition	Main effect Level	Condition x Level interaction	Condition x Group interaction	Level x Group interaction
RAVLT						
Acquisition	0.182	0.662	-	-	0.082	-
Learning rate	0.449	0.156	-	-	0.150	-
Proactive interference	0.828	0.418	-	-	0.070	-
Retroactive interference	0.761	0.403	-	-	0.996	-
Forgetting	0.589	0.625	-	-	0.119	-
Mental rotation test						
Response time	0.017*	0.418	0.006*	0.896	0.576	0.052
Accuracy	0.108	0.045*	<0.001*	0.778	0.147	0.787
Visual Search						
Response time	0.763	0.960	0.834	0.864	0.686	0.842
Accuracy	0.260	0.310	0.308	0.941	0.738	0.287
Stroop test						
Response time	0.485	0.145	0.109	0.840	0.274	0.702
Accuracy	0.261	0.310	0.308	0.941	0.739	0.287
RVIP						
Response time	0.374	0.466	-	-	0.836	-
True positive rate	0.475	0.886	-	-	0.152	-
Miss rate	0.484	0.888	-	-	0.156	-

Rapid Visual Information Processing, RVIP; Response time, RT; Rey Auditory Verbal Learning Test, RAVLT. *Indicates a significant effect ($P < 0.05$)
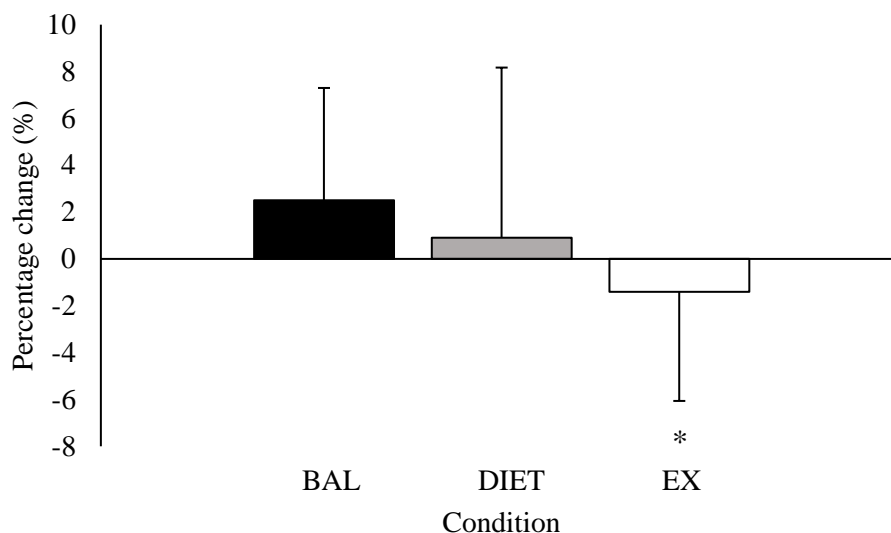
Figure 10.1. Mean ± 1SD percentage change in overall accuracy in the mental rotation test for controlled energy balance (BAL), dietary restriction (DIET) and exercise-induced energy restriction (EX). *Indicates a significant difference compared to BAL ($P < 0.05$).

10.3.2. Brunel Mood Scale

There was no effect of group on any component of the BRUMS score (main effect group, all $P > 0.05$) and group did not influence the response to each condition (group x condition interaction, all $P > 0.05$). The condition influenced the change from pre- to post-condition for Anger, Confusion and Fatigue (main effect condition, all $P < 0.05$). Post-hoc tests showed that whilst Anger increased in the DIET condition, it was reduced in the EX condition (BAL, +0.85; EX, -0.6; $P = 0.010$, $d = 0.85$; Figure 10.2) and was not different in BAL (-0.1) compared to DIET or EX conditions ($P > 0.05$). Confusion was reduced in the BAL condition and increased in the DIET condition (BAL, -0.75; DIET, +0.45; $P = 0.005$, $d = 0.46$), with EX (-0.3) not different to BAL or DIET conditions ($P > 0.05$). Fatigue was reduced in the BAL condition and increased in the EX condition (BAL, -0.9; EX, +1.45; $P = 0.027$, $d = 0.80$), while DIET (+0.65) was not different to BAL or EX ($P > 0.05$; Figure 10.2)

10.3.3. Pittsburgh Sleep Quality Index

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

167
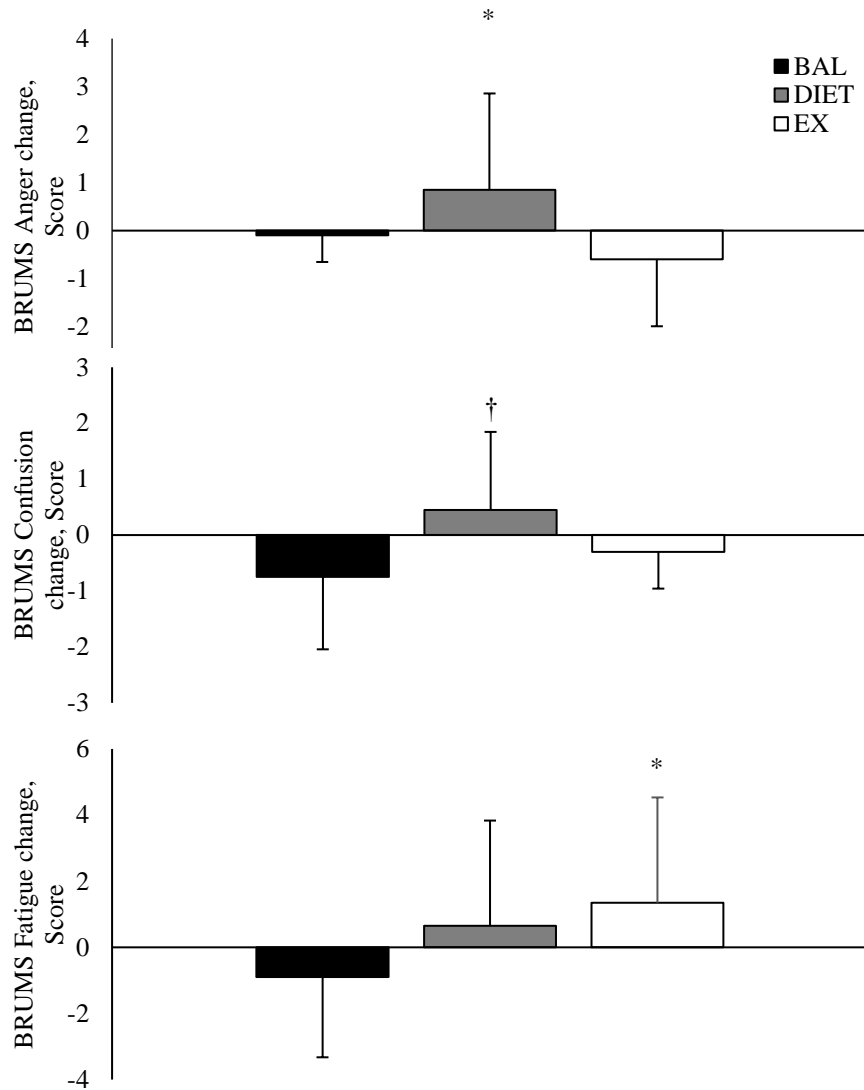
Figure 10.2. Mean ± 1SD change in score on the Brunel Mood Scale (BRUMS) from pre- to post-condition for controlled-energy balance (BAL), diet-induced energy restriction (DIET) and exercise-induced energy restriction (EX). *Indicates a significant difference to BAL ($P <$ 0.05) and † indicates a significant difference to EX ($P < 0.05$).

10.3.4. Oestradiol

Mean plasma oestradiol concentrations were significantly higher in eumenorrheic (143.0 ± 62.6 pmol·L^{-1}) than OC (51.2 ± 53.5 pmol·L^{-1}) participants (main effect group; $P < 0.001$, d = 1.58). There was a significant time x group interaction ($P < 0.001$); oestradiol concentrations were reduced from pre (73.8 ± 68.1 pmol·L^{-1}) to post (28.5 ± 11.2 pmol·L^{-1}; $P = 0.03$, d = 1.14) condition in OC participants and were not different from pre (125.4 ± 59.9 pmol·L^{-1}) to post (160.7 ± 61.1 pmol·L^{-1}) condition in eumenorrheic participants ($P = 0.129$, d = 0.58; Table 10.4). There was no effect of condition on oestradiol concentrations and condition did not interact with group or time ($P > 0.05$). These data are presented in Table 9.4.

168

10.4. Discussion

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

In line with previous research (Lieberman et al., 2008, 2017), we showed that for the majority of components of cognition measured, performance was not significantly affected by low energy availability, regardless of the method by which it was achieved. Previously, only grammatical reasoning and choice reaction time were shown to be adversely affected by low energy availability when achieved through severe calorie restriction and exercise (Lieberman et al. 2017). Whilst grammatical reasoning and choice reaction time were not directly measured in the current study, we found no effect of low energy availability (achieved via diet or exercise) on visual search test or Stroop test performance, which employ similar cognitive domains to choice reaction time. Furthermore, Lieberman et al. (2017) showed that in a small number of females (n = 6), working memory, grammatical reasoning and vigilance performance were superior in a calorie-restricted condition compared to an energy balanced condition, an effect that was not apparent in males. We did not measure grammatical reasoning, however in a larger sample of females (n = 20), we showed that working memory and vigilance performance were not affected by low energy availability, regardless of how it was achieved. These differences in findings may be due to less severe deviations from energy balance in the current study and differences in exercise intensity and duration. A higher exercise intensity was used for a shorter duration in the current study compared to previous research (Lieberman et al. 2008; 2017), whilst the dietary energy intakes were higher in both the exercise-induced and diet-induced energy restriction conditions. The current study was designed to differentiate between the effects of exercise and energy availability on cognitive function by including a non-exercise dietary restriction condition, exercise-induced low energy availability condition and a controlled-energy balance condition. Separating the effects of energy restriction from exercise is important as exercise can have significant effects on cognitive performance (*e.g.,* Chang et al., 2012; Tomporowski, 2003).

This is the first study to assess the effects of low energy availability on spatial awareness (as assessed by the mental rotation test) and our results showed that accuracy was significantly

impaired after exercise-induced energy restriction compared to the energy balanced condition, with no significant effects of diet-induced energy restriction on mental rotation performance (Figure 10.2). It is important to note that there was no interaction with test level (condition x level interaction), which suggests that this effect was not specific to higher rotation angles, and hence may not be specifically related to the mental operation of rotating visually presented objects. It is unclear why this cognitive domain was selectively impaired due to exercise-induced energy restriction, although this was a complex task, requiring the longest processing time (mean > 2 s) of the test battery in the current study and therefore may be more susceptible to interference.

The current study has improved upon previous research by not employing dietary placebos. In previous research (Lieberman et al., 2008, 2017), the calorie-restricted conditions consisted primarily of very low-calorie gels or non-nutritive foods to blind participants to the condition they were undertaking. Placebo-controlled designs are typically the gold-standard for randomised-controlled trials, however a series of studies have shown that dietary restraint, or the conscious effort to restrict energy intake, can impair cognitive performance (Green, Rogers, Elliman, & Gatenby, 1994; Kemps, Tiggemann, & Marshall, 2005; Rogers & Green, 1993), even in the absence of changes in weight (Green & Rogers, 1995). Therefore, the use of placebos is not an ecologically valid model, as it may negate some of the psychological consequences of consciously restricting energy intake, which would be present in real-world scenarios and may affect cognitive performance. The present study showed that using an ecologically valid model of low energy availability has minimal effects on cognitive function.

Between-group differences were observed for response time in the mental rotation test; OC user's performance improved to a greater extent from pre-to post-condition compared to eumenorrheic participants. The absence of a test level interaction (condition x level) in response time, also shows that this response was not likely to be specific to the rotational component and may also be a result of 'global' difference in response time. These effects occurred independently of changes in energy availability as they were apparent across all conditions, so it is likely that this was a result of differing reproductive hormone profiles. Endogenous oestradiol concentrations were significantly reduced from pre-to post-condition in the OC users and increased (d = 0.58, moderate), albeit non-significantly, from pre- to-post condition in the eumenorrheic participants. Mental rotation performance has been shown to be inversely related to oestrogen concentrations across the menstrual cycle, which may explain these findings (Hausmann et al., 2000; Silverman & Phillips, 1993) and this work provides further evidence of the importance of reproductive concentrations in spatial awareness. Alternatively, these results may be explained by the eumenorrheic participants sleep quality

being impaired over the 3 days compared to OC users, with sleep having been shown to consolidate performance gains in mental rotation performance (Debarnot, Piolino, Baron, Guillot, & Thiriet, 2013).

Overall, changes in energy availability had minimal effects of cognitive function, regardless of whether this was achieved through diet or exercise, in eumenorrheic women and OC users. Spatial awareness performance, however, was negatively affected only when exercise was used to induce a low energy availability, similar to quadriceps force results in Chapter 9. Irrespective of changes in energy availability, the change in mental rotation performance over the 3 days of each condition was different between OC users and non-users, evidencing the importance of reproductive hormones for this domain of cognition. This study provides evidence to support the concept that low energy availability may detrimentally affect aspects of physiological function other than reproductive function and bone metabolism, yet highlights the importance of considering reproductive status in this area (De Souza, et al., 2014; Mountjoy et al., 2014). This study has improved the current understanding of the effects of energy availability on cognitive performance by using ecologically valid methods of reducing energy availability, and a wide range of cognitive function tests.

Chapter 11.0. General discussion

11.1. Introduction

The health and performance of female athletes may be compromised with low energy availability and alterations to the reproductive axis. There has been controversy surrounding the physiological effects of reproductive hormones and low energy availability in female athletes, how these inter-relate and the physiological factors that are affected (De Souza et al., 2014; Mountjoy et al., 2014). The aims of this programme of work were to 1) Characterise the reproductive hormone models in elite female athletes by examining the prevalence of HC use, 2) Study the effects of the menstrual cycle and OC use on muscle function, cognition and bone metabolism, independent of changes in energy availability and, 3) Study the muscle function and cognitive responses to low energy availability, achieved through diet or exercise, and examine whether the responses are different in OC users and non-users.

11.2. Key Findings

- Quadriceps (ICC = 0.992, CV = 3.21%) and FDI muscle (ICC = 0.990, CV = 3.22%) MVIF measurements were shown to be reliable measures of muscle function.
- Approximately half of elite female athletes used HCs, with combined OC use the most prevalent and progestin-only contraceptives accounting for 30.0% of HC use.
- The side effects of the menstrual cycle and HC use were highly inter-individual.
- Combined HC users reported fewer perceived negative side effects compared to progestin-only HC users.
- Muscle force production, cognitive function and bone metabolism were not significantly affected by the menstrual cycle.
- Muscle force production and cognitive function were not different between phases of an OC cycle.
- Bone resorption marker (β-CTX) concentrations were significantly lower during late pill consumption compared to early pill consumption, while bone formation marker (P1NP) concentrations were not significantly different between OC phases.
- Diet-induced low energy availability did not affect muscle force production or cognitive function.
- Exercise-induced low energy availability impaired muscle force production in the exercised muscle only, and impaired mental rotation test performance.
- There were no differences in the muscle force or cognitive response to low energy availability between eumenorrheic women and OC users.
- Irrespective of low energy availability, OC user's mental rotation performance improved to a greater extent than eumenorrheic women over the course of three days of pill consumption/early follicular phase.

11.3. Prevalence of hormonal contraceptive use

In Chapter 5, it was shown that there was an approximately even distribution of HC users and non-users, evidencing that HC use is greater in the athlete population compared to the general public (~30%; Cea-Soriano, García Rodríguez, Machlitt, & Wallander, 2014; Daniels, Daugherty, & Jones, 2014). This may be due to non-contraceptive benefits of HCs such as the ability to manipulate menstruation around training and competition (Schaumberg et al., 2017) and reducing symptoms of dysmenorrhea (Ju, Jones, & Mishra, 2014), which may aid competitive performance (Chantler, Mitchell, & Fuller, 2009). This study provided important information regarding perceived side effects of HC use and the menstrual cycle in an elite athlete population, which had not previously been explored. The large inter-individual response in athletes highlights the need for coaches, practitioners and team doctors to open a dialogue with athletes on these issues so that individual needs of the athlete are met. This research has highlighted the wide range of HCs used by athletes (Figure 5.1), which needs to be considered in research to employ a representative sample of the female athlete population. Approximately 30% of HCs used are progestin-only preparations, although no research to date has studied the effects of these HCs on elements of athletic performance. Despite this, the majority (68.5%) of HC use was made up of combined OCs, and this study was the first to detail the preparations used by athletes that can be used to inform future research. Microgynon® was the most prevalent OC used and, therefore, this OC preparation was used in Chapters 6-8 to represent the most prevalent OC in female athletes, while incorporating rigorous experimental controls such as using one pill brand to reduce inter-individual variation (Elliott-Sale et al., 2013).

11.4. Muscle function

Muscle force production was not different between menstrual cycle phases or OC phases. This was despite endogenous oestrogen concentrations varying significantly between menstrual cycle phases and testing during early and late pill consumption and the PFI, which represent different exogenous hormone profiles (Kuhnz, Back, Power, Schütt, & Louton, 1991). The results for muscle function are in agreement with the majority of research in the area, albeit with an improved methodological approach compared to previous research, given the tight control of pre-test diet and exercise, measuring at three time-points of the menstrual and OC cycle and using a homogenous OC group. With the addition of the findings of this thesis, the totality of evidence, including research showing no effects of supra-physiological reproductive hormone concentrations on muscle force production (Elliott et al., 2005; Greeves, Cable, Luckas, Reilly, & Biljan, 1997) shows, that the reproductive hormone environment does not affect muscle force production. Furthermore, there was no difference in the muscle

function response to low energy availability between eumenorrheic women and OC users, indicating the reproductive hormone environment does not affect the muscle function response to short-term low energy availability. When low energy availability was induced through dietary restriction only, there was no effect on muscle force production, although exercise-induced low energy availability resulted in impaired quadriceps muscle force, without affecting FDI muscle force. Many athletes experience low energy availability due to expending large amounts of energy in exercise, with an inadvertent inability to match energy intake to energy demands (Nattiv et al., 2007). This thesis shows that muscle strength was reduced in the muscle group exercised to induce low energy availability, which may have implications for exercise performance and injury (Suchomel, Nimphius, & Stone, 2016) and provides evidence that this factor should be included in the RED-S model (Mountjoy et al., 2014). It is currently unclear, however, whether a similar decrement in muscle strength would be observed with the same exercise regimen in an energy-balanced condition. The lack of an effect of dietary-induced low energy availability on muscle force shows that it is not the low energy availability that impairs force, but rather the method by which this is achieved, which is not currently discussed as part of the Triad or RED-S models and should be considered moving forward.

11.5. Cognitive function

There was no difference in cognitive performance between phases of the menstrual cycle or OC cycle. While Chapter 7 showed that mental rotation accuracy was not different between menstrual cycle phases, mental rotation response time was not analysed due to a potential learning effect. In Chapter 10, however, it was shown that, irrespective of energy availability, mental rotation test response time was improved to a greater extent (-13.7%) from pre-to-post condition in OC users compared to eumenorrheic women (-4.0%). Between these time points, endogenous oestrogen was significantly reduced (-61.4%; d = 1.14) in OC users and increased (+28.1%; d = 0.58), albeit non-significantly with a medium effect size, in eumenorrheic women (condition x time interaction effect P < 0.05). The improved performance in mental rotation test performance when endogenous oestrogen was lower is in line with previous research across the menstrual cycle (Courvoisier et al., 2013; Hampson & Kimura, 1988; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Güntürkün, 2000; Maki, Rich, & Shayna Rosenbaum, 2002) and suggests that reproductive hormones may mediate this response. Whilst this research was not specifically designed to assess the effect of reproductive hormones, independent of energy availability, these results suggest that the reproductive hormone environment can acutely affect spatial awareness and this result occurred across energy-balanced (BAL) and low-energy availability (DIET, EX) conditions. This is in contrast

to bone health, where down-regulated reproductive hormones negatively affect bone metabolism. This shows that reduced reproductive hormone concentrations, as seen in hypothalamic amenorrhea, may be beneficial for spatial awareness performance, potentially mitigating any decrements in performance due to low energy availability. In fact, performance on the mental rotation test was the only cognitive measure that was affected by low energy availability. Accuracy improved (+2.5%) in the energy-balance condition, whilst there was a decline in the exercise-induced low energy availability condition (-1.4%). It is unclear why spatial awareness performance was selectively impaired, although it may be due to this test requiring the greatest cogitation, as evidenced by the longest mean response time (>2 s), thus making it more susceptible to interference. The decrement in performance was only evident in the exercise-induced low energy availability condition, which further highlights the need for models such as the Triad and RED-S to consider how the method by which low energy availability is achieved affects the physiological response.

11.6. Bone

Chapter 8 showed that there were no significant variations in bone formation or resorption across the menstrual cycle and bone formation was not significantly different across the menstrual cycle. Bone resorption was significantly greater during early pill consumption compared to late pill consumption. This was the first study to assess serum β-CTX and P1NP across the OC cycle in healthy women and provides evidence that changes in synthetic hormone concentrations influence bone metabolism. This highlights the importance of considering the effects of HC use on the bone metabolic responses to low energy availability, as current research has only studied eumenorrheic women (Ihle & Loucks, 2004; Papageorgiou et al., 2017), which represent a different reproductive hormone profile (Chapters 6-8). The bone metabolic responses to low energy availability were measured during data collection for Chapters 9 and 10 using the same study design, although these data are not presented in this thesis as this forms the work of a separate PhD student. Published work associated with these bone metabolic data are presented in Appendix 18 (Papageorgiou et al. 2018).

11.7. A critique of RED-S

The current RED-S model (Figure 2.6B) proposes that 20 separate factors related to health (n=10) and performance (n=10) may be negatively affected by low energy availability (Mountjoy et al., 2018). Whilst there is strong evidence for low energy affecting some of the proposed factors such as bone health (De Souza et al. 2014), menstrual function (Loucks et al. 2011), and endocrine function (Elliott-Sale et al., 2018), other purported health and

performance effects are not currently supported by a large amount of evidence. It is also not clear whether menstrual dysfunction also affects these factors, as seen in the Triad (De Souza et al., 2014). Judgement, co-ordination and concentration are factors that have been identified to potentially be affected by low energy availability in the RED-S model, although little evidence exists for this beyond self-reported decrements in athletes identified as being at risk of eating disorders (Ackerman et al., 2018). Furthermore, these terms are not clearly defined so it is difficult to objectively measure these factors at present. Spatial awareness, which was impaired with exercise-induced low energy availability, may span elements of co-ordination and judgement, providing some evidence for the inclusion of these factors in the RED-S model. In Chapter 10, however, there was no effect of low energy availability on RVIP task performance, a measure of concentration. This suggests that the cognitive aspects of RED-S may need re-consideration once more evidence is available, including the findings within the current thesis.

11.8. Limitations

1. In Chapters 6-8, the order participants completed experimental conditions was not counter-balanced. Cognitive function tests can display learning effects where performance is improved over time, so this may have affected the ability to interpret results. As part of the experimental protocol, participants completed a familiarisation session and were re-familiarised to computer-based cognitive tasks with practice stimuli prior to each test. Previous research has shown that there is a learning effect from first to second test presentation, with no further improvements after this (Falleti, Maruff, Collie, & Darby, 2006) and that pre-test practice stimuli reduce the occurrence of practice effects (Collie et al., 2003), although when analysed in chronological order of test administration there were learning effects for several tasks. The inability to differentiate the learning effects from effects of menstrual cycle phase or OC phase is recognised for the cognitive results in Chapter 7. This study measured cognitive performance at three time points of the menstrual cycle, in comparison to most previous research that has used two time points for cognitive research. Identifying participant ovulation and subsequent arrangement of ovulatory and mid-luteal test sessions caused scheduling issues that delayed these test sessions. Future research on cognitive function should employ a counter-balanced approach where possible.

2. In Chapters 9-10 a non-homogenous OC group was used, in contrast to recommendations from previous research (Elliott-Sale et al., 2013). This was unavoidable due to the need to access a wider pool of participants for recruitment, given the onerous nature of the study.

Whilst this may increase inter-individual variation, this provided a more representative sample of the population.

3. In Chapters 9-10, participants were regular exercisers, but were not athletes and, as such, were unaccustomed to the volume of exercise undertaken as part of the experimental design. Therefore, the response to this exercise regimen (*e.g.,* decline in quadriceps force) may have been greater in this population compared to athletes.

4. In Chapters 9-10, low energy availability was induced through dietary energy restriction or increased exercise-energy expenditure, providing a daily energy availability of 15 kcal·kgLBM^{-1}. Recent evidence has shown that within-day energy availability, for example the number of daily hours with energy balance < -300 kcal, may influence the metabolic response to low energy availability (Fahrenholtz et al. 2018), meaning that daily estimates of energy availability may lack some sensitivity. Chapters 9-10 were not designed to assess the magnitude of within-day energy deficiencies, however future research should assess this were possible.

11.9. Future directions

- The work described in this thesis studied the acute response to changes in reproductive hormones and energy availability. Future research should explore the muscle function and cognitive response to long-term (*e.g.,* months to years) changes to the reproductive hormone environment and/or low energy availability as this may alter physiological function via different mechanisms such as structural adaptations, which do not occur in the short-term.

- Chapter 5 identified that progestin-only HCs account for 30% of HCs used by elite female athletes, yet no research to date has studied the effects of these HCs on athletic performance. Future research should explore any potential effects, focussing on the most prevalent delivery methods such as progestin-only OCs and implants.

- In Chapters 9-10, eumenorrheic women were studied in the early follicular phase which represents the lowest concentration of endogenous reproductive hormones, which are most similar to OC users. Future research should explore differences in responses between eumenorrheic women and OC users at phases of the menstrual cycle where oestrogen concentrations are higher such as the mid-luteal phase. Similarly, OC users were studied during the first week of pill consumption. Future research should study OC users during late pill consumption, when exogenous reproductive hormone concentrations are higher, or during the PFI, when no

exogenous hormones are supplied to represent different reproductive hormone environments.

11.10. Conclusions

The RED-S model suggests that muscle strength and elements of cognitive function such as decreased concentration, decreased co-ordination and impaired judgement may be affected by reduced energy availability (Mountjoy et al., 2014). The current work has shown that, in the short-term, low energy availability does not affect muscle strength or a wide range of cognitive functions, unless the low energy availability is induced by exercise, in which case only the exercised muscle and spatial performance are selectively impaired. Future iterations of the RED-S model should consider how the method by which low energy is achieved affects RED-S outcomes. Furthermore, muscle strength and cognitive function were not affected by menstrual cycle or OC phase and there was no difference in the response to low energy availability for muscle strength and cognitive function between eumenorrheic women and OC users. This suggests that changes to the reproductive hormone environment with low energy availability may not synergistically affect muscle function and cognitive function, in the same way that hypothalamic amenorrhea exacerbates negative bone metabolic outcomes with low energy availability (De Souza et al., 2008; De Souza et al., 2014). The evidence that bone resorption is significantly different between OC phases provides further support for the importance of reproductive hormones in regulating bone metabolism and highlights the importance of considering how HC use may influence bone metabolic outcomes with low energy availability, which are not considered in the Triad or RED-S models, despite the current work showing that half of athletes used HCs.

Chapter 12.0. References

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Chapter 13.0. Appendices

Appendix 1. Rey Auditory Verbal Learning Test (RAVLT) word lists used in Chapter 7 and 10

RAVLT SET 1

Name _____

Date_____

Examiner_____

(Note: Do not-re-read List A for Recall Trial A6 or A7										
List A	*A1*	*A2*	*A3*	*A4*	*A5*	*List B*	*B1*	*A6*	*A7*	
Drum						Desk				Drum
Curtain						Ranger				Curtain
Bell						Bird				Bell
Coffee						Shoe				Coffee
School						Stove				School
Parent						Mountain				Parent
Moon						Glasses				Moon
Garden						Towel				Garden
Hat						Cloud				Hat
Farmer						Boat				Farmer
Nose						Lamb				Nose
Turkey						Gun				Turkey
Colour						Pencil				Colour
House						Church				House
River						Fist				River
# Correct										

RAVLT SET 2

Name _____

Date_____

Examiner_____

(Note: Do not-re-read List A for Recall Trial A6 or A7									
List A	*A1*	*A2*	*A3*	*A4*	*A5*	*List B*	*B1*	*A6*	*A7*	
Pipe						Bench				Pipe
Wall						Officer				Wall
Alarm						Cage				Alarm
Sugar						Sock				Sugar
Student						Fridge				Student
Mother						Cliff				Mother
Star						Bottle				Star
Painting						Soap				Painting
Bag						Sky				Bag
Wheat						Ship				Wheat
Mouth						Goat				Mouth
Chicken						Bullet				Chicken
Sound						Paper				Sound
Door						Chapel				Door
Stream						Crab				Stream
# Correct										

RAVLT SET 3

Name _____

Date_____

Examiner_____

(Note: Do not-re-read List A for Recall Trial A6 or A7										
List A	*A1*	*A2*	*A3*	*A4*	*A5*	*List B*	*B1*	*A6*	*A7*	
Violin						Orange				Violin
Tree						Table				Tree
Scarf						Toad				Scarf
Ham						Corn				Ham
Suitcase						Bus				Suitcase
Cousin						Chin				Cousin
Earth						Beach				Earth
Stairs						Soap				Stairs
Dog						Hotel				Dog
Banana						Donkey				Banana
Town						Spider				Town
Radio						Monkey				Radio
Hunter						Book				Hunter
Bucket						Soldier				Bucket
Field						Padlock				Field
# Correct										

RAVLT SET 4

Name _____

Date_____

Examiner_____

(Note: Do not-re-read List A for Recall Trial A6 or A7									
List A	*A1*	*A2*	*A3*	*A4*	*A5*	*List B*	*B1*	*A6*	*A7*	
Arm						Boot				Arm
Cat						Monkey				Cat
Axe						Bowl				Axe
Bed						Cow				Bed
Place						Finger				Place
Ear						Dress				Ear
Dog						Spider				Dog
Hammer						Cup				Hammer
Chair						Bee				Chair
Car						Foot				Car
Eye						Hat				Eye
Horse						Butterfly				Horse
Knife						Kettle				Knife
Clock						Mouse				Clock
Bike						Hand				Bike
# Correct										

RAVLT SET 5

Name _____

Date_____

Examiner_____

(Note: Do not-re-read List A for Recall Trial A6 or A7										
List A	*A1*	*A2*	*A3*	*A4*	*A5*	*List B*	*B1*	*A6*	*A7*	
Fox						Sandwich				Fox
Toaster						Mechanic				Toaster
Letter						Pot				Letter
Liver						Stove				Liver
Train						Phone				Train
Uncle						Lawyer				Uncle
Lion						Tablet				Lion
Helicopter						Slipper				Helicopter
Child						Clothes				Child
Badger						Pasta				Badger
Nephew						Teacher				Nephew
Chest						Ring				Chest
Hair						Watch				Hair
Window						Drain				Window
Tube						Peach				Tube
# Correct										

RAVLT SET 6

Name _____

Date_____

Examiner_____

(Note: Do not-re-read List A for Recall Trial A6 or A7										
List A	*A1*	*A2*	*A3*	*A4*	*A5*	*List B*	*B1*	*A6*	*A7*	
Key						Pint				Key
Worm						Station				Worm
Light						Water				Light
Bolt						Meter				Bolt
Hen						Rice				Hen
Writer						Amber				Writer
Butcher						Bank				Butcher
Wrench						Black				Wrench
Tin						Oil				Tin
Wood						Plum				Wood
Snake						Toast				Snake
Pillow						Navy				Pillow
Plastic						Liquid				Plastic
Doctor						Gym				Doctor
Screw						Yard				Screw
# Correct										

Appendix 2. Profile of Moods State (POMS) questionnaire used in Chapter 7

PROFILE OF MOODS QUESTIONNAIRE

POMS is a standard validated psychological test. The questionnaire contains 65 words/statements that describe feelings people have. The test requires you to indicate for each word or statement how you have been feeling in the past week including today.

Instructions:

Read each word/statement below, decide how you have been feeling, in respect to the word/statement, in the past week and today, and circle the number corresponding to the appropriate statement "Not at All", "A Little", "Moderately", "Quite a Lot" or "Extremely" to indicate your feeling. There is no time limit.

Statement	Not at all	A little	Moderately	Quite a lot	Extremely
Friendly	1	2	3	4	5
Tense	1	2	3	4	5
Angry	1	2	3	4	5
Worn out	1	2	3	4	5
Unhappy	1	2	3	4	5
Clearheade	1	2	3	4	5
Lively	1	2	3	4	5
Confused	1	2	3	4	5
Sorry for thing done	1	2	3	4	5
Shaky	1	2	3	4	5
Listless	1	2	3	4	5
Peeved	1	2	3	4	5
Considerate	1	2	3	4	5
Sad	1	2	3	4	5
Active	1	2	3	4	5
On edge	1	2	3	4	5
Grouchy	1	2	3	4	5
Blue	1	2	3	4	5
Energetic	1	2	3	4	5
Panicky	1	2	3	4	5
Hopeless	1	2	3	4	5
Relaxed	1	2	3	4	5
Unworthy	1	2	3	4	5
Spiteful	1	2	3	4	5
Sympatheti	1	2	3	4	5
Uneasy	1	2	3	4	5
Restless	1	2	3	4	5
Unable to	1	2	3	4	5
Fatigue	1	2	3	4	5
Helpful	1	2	3	4	5

Statement	Not at all	A little	Moderately	Quite a lot	Extremely
Annoyed	1	2	3	4	5
Discouraged	1	2	3	4	5
Resentful	1	2	3	4	5
Nervous	1	2	3	4	5
Lonely	1	2	3	4	5
Miserable	1	2	3	4	5
Muddled	1	2	3	4	5
Cheerful	1	2	3	4	5
Bitter	1	2	3	4	5
Exhausted	1	2	3	4	5
Anxious	1	2	3	4	5
Ready to fight	1	2	3	4	5
Good	1	2	3	4	5
Gloomy	1	2	3	4	5
Desperate	1	2	3	4	5
Sluggish	1	2	3	4	5
Rebellious	1	2	3	4	5
Helpless	1	2	3	4	5
Weary	1	2	3	4	5
Bewildered	1	2	3	4	5
Alert	1	2	3	4	5
Deceived	1	2	3	4	5
Furious	1	2	3	4	5
Efficient	1	2	3	4	5
Trusting	1	2	3	4	5
Full of pep	1	2	3	4	5
Bad	1	2	3	4	5
Worthless	1	2	3	4	5
Forgetful	1	2	3	4	5
Carefree	1	2	3	4	5
Terrified	1	2	3	4	5
Guilt	1	2	3	4	5
Vigorous	1	2	3	4	5
Uncertain	1	2	3	4	5
Bushed	1	2	3	4	5

Appendix 3. Brunel Mood State (BRUMS) questionnaire used in Chapter 10

BRUNEL MOOD SCALE QUESTIONNAIRE

BRUMS is a standard validated psychological test. The questionnaire contains 24 words that describe feelings people have. The test requires the participant to indicate for each word(s) or statement how you have been feeling in the past week including today.

Instructions: Below is a list of words that describe feelings. Please read each one carefully. Then tick the box which describes *how you feel right now*. Make sure you answer every question. There is no time limit.

Word(s)	Not at all	A little	Moderately	Quite a lot	Extremely
Panicky					
Lively					
Confused					
Worn out					
Depressed					
Downhearted					
Annoyed					
Exhausted					
Mixed-up					
Sleepy					
Bitter					
Unhappy					
Anxious					
Worried					
Energetic					
Miserable					
Muddled					
Nervous					
Angry					
Active					
Tired					
Bad tempered					
Alert					
Uncertain					

Appendix 4. Pittsburgh Sleep Quality Index (PSQI) questionnaire used in Chapters 7 and 10

PITTSBURGH SLEEP QUALITY INDEX

SUBJECT'S INITIALS ID#   DATE   TIME   AM /
          PM

INSTRUCTIONS:
THE FOLLOWING QUESTIONS RELATE TO YOUR USUAL SLEEP HABITS DURING THE PAST WEEK ONLY. YOUR ANSWERS SHOULD INDICATE THE MOST ACCURATE REPLY FOR THE MAJORITY OF DAYS AND NIGHTS IN THE PAST WEEK. PLEASE ANSWER ALL QUESTIONS.

1. DURING THE PAST WEEK, WHAT TIME HAVE YOU USUALLY GONE TO BED AT NIGHT?
                    BED TIME _____

2. DURING THE PAST WEEK, HOW LONG (IN MINUTES) HAS IT USUALLY TAKEN YOU TO FALL ASLEEP EACH NIGHT?
                    NUMBER OF MINUTES _____

3. DURING THE PAST WEEK, WHAT TIME HAVE YOU USUALLY GOTTEN UP IN THE MORNING?
                    GETTING UP TIME _____

4. DURING THE PAST WEEK, HOW MANY HOURS OF ACTUAL SLEEP DID YOU GET AT NIGHT? (THIS
MAY BE DIFFERENT THAN THE NUMBER OF HOURS YOU SPENT IN BED.)
                    HOURS OF SLEEP PER NIGHT _____

FOR EACH OF THE REMAINING QUESTIONS, CHECK THE ONE BEST RESPONSE. PLEASE ANSWER ALL QUESTIONS.

5. DURING THE PAST WEEK, HOW OFTEN HAVE YOU HAD TROUBLE SLEEPING BECAUSE YOU . . .

A) CANNOT GET TO SLEEP WITHIN 30 MINUTES
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

B) WAKE UP IN THE MIDDLE OF THE NIGHT OR EARLY MORNING
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

C) HAVE TO GET UP TO USE THE BATHROOM
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

D) CANNOT BREATHE COMFORTABLY
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

E) COUGH OR SNORE LOUDLY
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

F) FEEL TOO COLD
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

G) FEEL TOO HOT
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

H) HAD BAD DREAMS
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

I) HAVE PAIN
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

J) OTHER REASON(S), PLEASE DESCRIBE_____
HOW OFTEN DURING THE PAST WEEK HAVE YOU HAD TROUBLE SLEEPING BECAUSE OF THIS?
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

6. DURING THE PAST WEEK, HOW WOULD YOU RATE YOUR SLEEP QUALITY OVERALL?
- VERY GOOD _____
- FAIRLY GOOD _____
- FAIRLY BAD _____
- VERY BAD _____

7. DURING THE PAST WEEK, HOW OFTEN HAVE YOU TAKEN MEDICINE TO HELP YOU SLEEP (PRESCRIBED OR "OVER THE COUNTER")?
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

8. DURING THE PAST WEEK, HOW OFTEN HAVE YOU HAD TROUBLE STAYING AWAKE WHILE DRIVING, EATING MEALS, OR ENGAGING IN SOCIAL ACTIVITY?
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

9. DURING THE PAST WEEK, HOW MUCH OF A PROBLEM HAS IT BEEN FOR YOU TO KEEP UP ENOUGH ENTHUSIASM TO GET THINGS DONE?

NO PROBLEM AT ALL _____

ONLY A VERY SLIGHT PROBLEM _____

SOMEWHAT OF A PROBLEM _____

A VERY BIG PROBLEM _____

10. DO YOU HAVE A BED PARTNER OR ROOM MATE?

NO BED PARTNER OR ROOM MATE _____

PARTNER/ROOM MATE IN OTHER ROOM _____

PARTNER IN SAME ROOM, BUT NOT SAME BED _____

PARTNER IN SAME BED _____

IF YOU HAVE A ROOM MATE OR BED PARTNER, ASK HIM/HER HOW OFTEN IN THE PAST WEEK YOU HAVE HAD . . .

A) LOUD SNORING
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

B) LONG PAUSES BETWEEN BREATHS WHILE ASLEEP
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

C) LEGS TWITCHING OR JERKING WHILE YOU SLEEP
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

D) EPISODES OF DISORIENTATION OR CONFUSION DURING SLEEP
- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

E) OTHER RESTLESSNESS WHILE YOU SLEEP; PLEASE DESCRIBE

_____

_____

- NOT DURING THE PAST WEEK _____
- ONCE OR TWICE A WEEK_____
- THREE OR MORE TIMES A WEEK_____

Thank you for completing this questionnaire

Appendix 5. International Physical Activity Questionnaire (IPAQ) used in Chapters 9 and 10

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

Name:

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**.

Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**
No vigorous physical activities *Skip to question 3*

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**
_____ **minutes per day**
Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**
No moderate physical activities *Skip to question 5*

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**
_____ **minutes per day**
Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**
No walking *Skip to question 7*

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**
_____ **minutes per day**
Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**
_____ **minutes per day**
Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix 6. Participant information sheet used in Chapter 4

"Determination of the reliability of the first dorsal interosseus and quadriceps maximal isometric strength."

Brief Introduction:

It is important for researchers to know the reliability of the tests which they use. By understanding the day to day variation in performance for each test, we can then identify how easy it will be to detect a change in performance due to an intervention or change. Therefore, this study aims to assess the repeatability of two performance tests

Study Requirements:

You will be required to visit the laboratory on two occasions, separated by 24 hours. Each session will last a total of 40 minutes.

Location:

Erasmus Darwin Building room 256 (ERD256) at Nottingham Trent University

Restrictions during testing:

In the 24 hours prior to the first testing session you will be asked refrain from vigorous exercise and make a note of habitual exercise (i.e. cycling to work) and caffeine intake. You will then be asked to replicate this in the following 24 hours before the second visit to the laboratory.

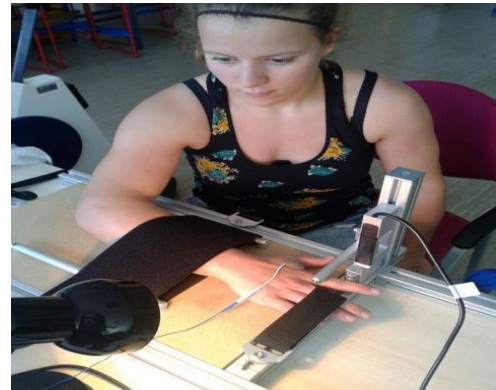Figure 1. Assessment of quadriceps maximal force production

Figure 2. Assessment of first dorsal interosseus maximal force production

Testing Protocol:

- Height, weight, date of birth and contraceptive use information (if applicable) will be taken
- You will perform a 5 minute light warm-up on a cycle ergometer
- You will then be seated in a custom-built dynamometer (Figure 1) where we will measure the force you can produce when attempting to extend (straighten) your leg whilst your ankle is cuffed into a fixed position.
- After this, we will ask you to place your right hand in a water bath until your hand temperature is raised to 40°

We will then measure the force you can produce with your first dorsal interosseus (muscle which moves your index finger towards your thumb) on a dynamometer (Figure 2)

Potential Risks to You

- Muscle soreness may be experienced during and in the days following the assessments

You are free to withdraw from testing at any time without explanation.

Contacts:

Mr Daniel Martin: 07773466063
Daniel.Martin@ntu.ac.uk

Appendix 7. Informed consent form used in Chapter 4

<u>Subject Statement of Consent to Participate in the Investigation Entitled:</u>

"Determination of the reliability of the first dorsal interosseus and quadriceps maximal isometric strength"

1) I, _____ agree to partake as a subject in the above study.

2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with *Daniel Martin* that this will involve me: *Attending the Sport Science laboratories at NTU Clifton Campus on 2 different occasions, with each visit lasting up to 40 minutes. I will be required undergo muscle function testing.*

3) It has also been explained to me by *Daniel Martin* that the risks and side effects which may result from my participation are as follows: *Muscle soreness may occur in the days following the test.*

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent to participate in the study at any time and for any reason, without having to explain my withdrawal.

7) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.

8) I confirm that I have read the University's policy relating to the storage and subsequent destruction of sensitive information. I understand that sensitive information I have provided through my participation in this study, will be handled in accordance with this policy.

9) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

Subject signature: ……………………………………………………………
Date:_____

Independent witness signature: ………………………………………………
Date:_____

Primary Researcher signature: ………………………………………………
Date:_____

Appendix 8. Health Screen used in Chapters 4 and 6 to 10

HEALTH SCREEN

Name or Number   ………………..………………

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a)   on medication, prescribed or otherwise   Yes ☐   No ☐

(b)   attending your general practitioner   Yes ☐   No ☐

(c)   on a hospital waiting list   Yes ☐   No ☐

2. **In the past two years,** have you had any illness which require you to:

(a)   consult your GP   Yes ☐   No ☐

(b)   attend a hospital outpatient department   Yes ☐   No ☐

(c)   be admitted to hospital   Yes ☐   No ☐

3. **Have you ever** had any of the following?

(a)   Convulsions/epilepsy   Yes ☐   No ☐

(b)   Asthma   Yes ☐   No ☐

(c)   Eczema   Yes ☐   No ☐

(d)   Diabetes   Yes ☐   No ☐

(e)   A blood disorder   Yes ☐   No ☐

(f)   Head injury   Yes ☐   No ☐

(g)   Digestive problems   Yes ☐   No ☐

(h)   Heart problems   Yes ☐   No ☐

(i)   Problems with bones or joints   Yes ☐   No ☐

(j)   Disturbance of balance / coordination   Yes ☐   No ☐

(k)   Numbness in hands or feet   Yes ☐   No ☐

(l)   Disturbance of vision   Yes ☐   No ☐

(m)   Ear / hearing problems   Yes ☐   No ☐

(n)   Thyroid problems   Yes ☐   No ☐

(o)   Kidney or liver problems   Yes ☐   No ☐

(p)   Allergy to nuts, alcohol etc.   Yes ☐   No ☐

(q)   Any problems affecting your nose e.g. recurrent nose bleeds   Yes ☐   No ☐

(r)   Any nasal fracture or deviated nasal septum   Yes ☐   No ☐

4. **Has any,** otherwise healthy, member of your family under the age of 50
    died suddenly during or soon after exercise?   Yes ☐ No ☐

5. Are there any reasons why blood sampling may be difficult?   Yes ☐ No ☐

6. Have you had a blood sample taken previously?   Yes ☐ No ☐

7. Have you had a cold, flu or any flu like symptoms in the last month?   Yes ☐ No ☐

Appendix 9. Participant information sheet used in Chapter 5

Participant information sheet

'The prevalence of hormonal contraceptive use in female athletes'

Background

Hormonal contraceptives are any form of contraceptive that primarily works by releasing hormones (oestrogens and/or progestins) into the body to alter the hormone profile and prevent pregnancy. There are many types of hormonal contraceptive including the oral contraceptive, injection, implant, intrauterine device (coil), vaginal ring and transdermal patch. Each type of hormonal contraceptive has different brands which deliver different types and concentrations of oestrogens and progestins into the system. Although it is estimated that approximately 40-50% of female athletes use the oral contraceptive pill, the brands of oral contraceptive have not been reported and data are not available for the prevalence of other types of hormonal contraception in female athletes. There is evidence that hormonal contraceptives may influence body composition and alter the response to training, in addition to acutely affecting exercise performance. These effects may be positive or negative depending on the types of oestrogens and/or progestins contained within the hormonal contraception so it is important to understand the current use of hormonal contraceptives in female athletes in more detail.

The aim of this study is to determine the prevalence of hormonal contraceptive use (and non-use) in female athletes.

Why have I been invited to participate in this study?

You have been asked to complete this questionnaire as you have been identified as a female athlete competing at a high-level. To take part in this study you must be at least 18 years of age.

What will I have to do?

After reading this information sheet carefully, you will be asked to complete the informed consent form provided to confirm that you are aware of what the study involves and agree to participate.

Once you have completed the informed consent form, please complete the questionnaire in as much detail as possible and place the informed consent form and questionnaire in the envelope provided, re-seal it using the double sided tape attached to the envelope and hand it back to your coach who will send it to our research team.

Will my participation in this study be kept confidential?

Yes. The data you provide will be kept entirely confidential.

What if I want to withdraw myself from the study?

You can withdraw your data from this study in the 3 months following completion of the questionnaire. In order for you to do this, you will be asked to provide a 'unique identifier' on the questionnaire which acts as a password so we can identify your particular data set to remove it from our results if you wish. Please contact the research team using the details provided if you would like us to do this.

Potential risks to you

There are no risks associated with taking part in this study.

Potential benefit to you

By helping to understand the prevalence of hormonal contraceptive use in female athletes you are increasing the knowledge in this area which will benefit female athletes by targeting future research to the needs of this population.

Contact Details

Investigator:

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NG11 8NS

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Investigator:

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NG11 8NS

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Phone: +44 (0)115 848 6338

Appendix 10. Informed consent form used in Chapter 5

NOTTINGHAM
TRENT UNIVERSITY

Participant Consent Form

I (participant name) …………………………………………………… have read the participant information sheet provided and agree to participate in this project studying the prevalence of hormonal contraceptive use in female athletes and I confirm that I am over the age of 18.

I have carefully read the information sheet and understand that I am required to complete the questionnaire provided. I understand that it is possible to withdraw my consent to participate in the research within 3 months of completing the questionnaire without any obligation to explain why. I also realise that any personal information regarding myself will be confidential.

Signed (Participant)..   Date ………………………

Appendix 11. Questionnaire used in Chapter 5

Hormonal contraceptive use in female athletes

Please complete this questionnaire as accurately and as thoroughly as possible. All data collected from this questionnaire is confidential and anonymised. You have the right to withdraw your data at any time. In the case you wish to withdraw your data from the study we require a way of identifying your responses as it will be stored in anonymised form. Please provide a word or phrase which can be used as your unique identifier (i.e. first pets name, mothers' maiden name or a significant event etc.) in order to identify your data if you would like it to be withdrawn.

Unique identifier (optional):_____

General information

Date of Birth:_____

Height (approximate in meters or feet and inches):_____

Weight (approximate in kilograms or stones and pounds):_____

Competition and training

1. What sport/event do you primarily compete in?_____

2. What level do you currently compete at (i.e. regional, national, premier division, international, ranking)? Please provide as much detail as possible relevant to your sport: _____

_____

3. How long have you competed at this level?_____

4. How many training sessions do you undertake in an average week?_____

5. How long are these training sessions on average (minutes) ?_____

Section A: Overview of reproductive functioning

1. What was your approximate age at menarche (first period)?_____

Types of hormonal contraceptives include
- Oral contraceptives
- Implant
- Injection
- Intrauterine device/coil (hormone releasing)
- Vaginal ring
- Contraceptive (transdermal) patch

2. Do you currently use any type of hormonal contraceptive?   YES / NO

If NO, please proceed to Section B

If YES, please proceed to Section C

Section B: Menstrual cycle characteristics

1. Do you use a non-hormonal intrauterine device (copper-based coil)?

    YES / NO

2. Approximately, what is the total length of your menstrual cycle, from day 1 of your period, to the next day 1 of your period?_____

3. Is the length of your menstrual cycle variable?   YES / NO

    If the answer was YES, please state how much your cycle typically varies by (e.g. 28-35 days):_____

4. Do you get pain or other symptoms during your menstrual cycle (e.g. headaches, stomach cramps)?   YES / NO

    If YES, please state the symptoms and at what point during your cycle you suffer these:_____

5. Do you avoid exercise/training during any point of your menstrual cycle? YES / NO

    If YES, please state when during your menstrual cycle and state the reasons why you avoid exercise/training:_____

_____

6. Do you take any other medication?   YES / NO

    If YES, please state what medication you use and how often:_____

_____

Please proceed to Section D

Section C: Current hormonal contraceptive use

Types of hormonal contraceptives include
- Oral contraceptives
- Implant
- Injection
- Intrauterine device/coil (hormone releasing)
- Vaginal ring
- Contraceptive (transdermal) patch

1. Which type of hormonal contraception do you currently use?_____
_____

2. What is the brand name of this contraception (See below)?_____
_____

There are different brands within each type of contraception which contain different types and amounts of hormones so it is important that we know which brand is used. This might be the name on the packet of pills (i.e. Microgynon 30® or Cilest®), the name of the intrauterine device (i.e. Mirena® coil), the name of the contraceptive implant (i.e. Norplant®), the name of the injection (i.e. Depo Provera®), the name of the vaginal ring (i.e. NuvaRing®) or the name of the contraceptive patch (i.e. Ortho Evra®).

3. How long (years/months) have you used this method of contraception?_____
_____

4. Have you discussed your hormonal contraceptive use with your coach/team doctor etc.?   YES / NO

5. Was your coach/team doctor involved in the decision to use this type of hormonal contraceptive?

YES / NO

      If YES, why did you choose this type of contraceptive?_____
_____

6. When deciding whether or not to use this method of hormonal contraception, did you consider possible side effects? YES / NO

If YES, what side effects were these:_____

_____

7. Have you received any negative side effects from use of this method of contraception? YES / NO

If YES, what side effects:_____

_____

8. Were there any positive effects of hormonal contraceptive use (other than preventing pregnancy), that you considered when opting for hormonal contraceptive use? YES / NO

If YES, what were

these:_____

_____

9. Were there any particular reasons you chose to use this method of hormonal contraceptive over other methods? YES / NO

If YES, please state these reasons:_____

_____

10. Do you take any other form of medication?   YES / NO

If YES, please state what medication you use:_____

_____

Please Proceed to Section D

Section D: Previous use of hormonal contraceptives

Types of hormonal contraceptives include

- Oral contraceptives
- Implant
- Injection
- Intrauterine device/coil (hormone releasing)
- Vaginal ring
- Contraceptive (transdermal) patch

In the past, have you used (*other*) forms of hormonal contraception?

YES / NO

If YES, please state the type and brand (see below) the duration of use and the reason you stopped using any previous hormonal contraceptives in the table below:

There are different brands within each type of contraception which contain different types and amounts of hormones so it is important that we know which brand is used. This might be the name on the packet of pills (i.e. Microgynon 30® or Cilest®), the name of the intrauterine device (i.e. Mirena® coil), the name of the contraceptive implant (i.e. Norplant®), the name of the injection (i.e. Depo Provera®), the name of the vaginal ring (i.e. NuvaRing®) or the name of the contraceptive patch (i.e. Ortho Evra®).

Type	Brand	Duration of use	Reason you stopped using it

Thank you for completing this Questionnaire

Please place the questionnaire in the envelope provided, seal the envelope (using the double sided tape attached to the envelope) and hand it back to your coach

Appendix 12. Menstrual cycle characteristics questionnaire used in Chapters 6 to 10

MENSTRUAL CYCLE DETAILS

(All information is fully confidential)

Please circle the answer where appropriate.

Name:

Age:

Date of birth:

1) Have you had regular periods in the last six months?　　　YES　　　　　　NO

2) How long in days is your menstrual cycle, from day 1 of bleeding (period) to day 1 of the next period?

_____DAYS

3) Is the above time the same between periods?　　　YES　　　　　　NO

If the answer was NO, please state the irregularity:

_____
_____
_____

4) How many days does your menstrual (blood) flow last?

_____DAYS

5) Do you get pain during your period?　　　YES　　　　　　NO

If YES, please state the symptoms and the days during the cycle when you suffer:

_____
_____
_____

6) Do you avoid exercise during your period?　　　YES　　　　　　NO

If YES, please state your reasons for avoiding exercise:

_____
_____
_____

7) Do you take any medication or hormones to regulate your menstrual cycle?

NO   YES

If YES, please state what you take and how often?

_____
_____
_____

8) Do you take any other medication?

NO   YES

If YES, please state what you take and how often?

_____
_____
_____

9) Have you previously used any form of hormonal contraception (oral contraceptive, implant, injection, coil)?

NO   YES

If YES, please state the type of contraception used and the date that you ceased using it?

_____
_____
_____

10) When did you have your last period (day 1)?

_____

Appendix 13. Oral contraceptive use questionnaire used in Chapters 6 to 10

ORAL CONTRACEPTIVE DETAILS

(All information is fully confidential)

Please circle the answer where appropriate.

Name:

Age:

Date of birth:

1) What brand or oral contraceptive do you take?_____

2) How long have you been using your current oral contraceptive?_____

3) What date did you begin taking your current pack of pills?_____

4) What time of day do you usually consume the pill?_____

5) Will you be continuing to take the oral contraceptive for the next 2 months?

      NO   YES

6) Do you take any other medication or hormones to regulate your cycle?

      NO   YES

If YES, please state what you take and how often?

_____

_____

_____

7) Do you take any other medication?

      NO   YES

If YES, please state what you take and how often?

_____

_____

_____

Appendix 14. Participant information sheet used in Chapters 6 to 8

PARTICIPANT INFORMATION SHEET

"The effects of menstrual cycle phase and oral contraceptive use on energy expenditure, bone turnover, cognitive function and muscle function in females."

Brief Introduction:
Over the course of a menstrual cycle there are cyclical changes in the concentrations of various hormones including oestrogen and progesterone, however with oral contraceptive use the concentrations of these hormones remain relatively stable. It is important to understand how the changes in these hormones during the menstrual cycle and oral contraceptive use affects measures of performance and health. Therefore, the aim of this study is to assess if there are changes in muscle function, cognitive function, energy expenditure and bone metabolism over the duration of one menstrual cycle or one oral contraceptive cycle.

Study Requirements:
You will be required to visit the laboratory on 5 occasions; one introductory session which will last approximately 40 minutes, one familiarisation session and three main test sessions which will all be identical and each last approximately 2 hours. The three main test sessions will be completed on days 3, 15 and 23 of your menstrual cycle or oral contraceptive cycle. All test sessions will begin at 8.30am and before each visit to the laboratory we will contact you with specific information regarding the dates you are required to come into the laboratory.

Location:
Erasmus Darwin Building room 256 (ERD256) at Nottingham Trent University
Clifton Campus, Clifton Lane, NG11 8NS.

Restrictions during testing:
For each visit to the laboratory you will be required to fast from 10pm the previous night, with the exception of water. In the days preceding each visit to the laboratory you will be asked to replicate your diet and record your physical activity. In addition, you will be asked to avoid strenuous exercise and alcohol consumption in the 24h prior to a test session, however throughout the remainder of the study you can maintain your normal diet and activity levels.
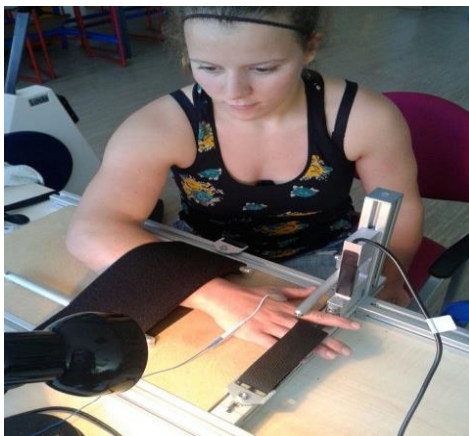

Figure 1. Assessment of first dorsal interosseus maximal force production

Figure 2. Assessment of quadriceps maximal force production

Testing Protocol:

Introductory session
- We will explain the testing procedures in detail.
- You will be asked to complete a menstrual history questionnaire.
- We will measure your body composition using skinfold callipers, cross-sectional area of the first dorsal interosseus (muscle in the hand that moves your index finger towards your thumb) using ultrasonography and height and weight.

Familiarisation and main test sessions
- Arrive to the laboratory at 8.30am for a fasted blood sample after consuming 600ml water.
- After 20 minutes of rest we will collect a 5 minute sample of expired air which we will use to estimate your resting energy expenditure.
- You will then be asked to complete standardised questionnaires including the Pittsburgh Sleep Quality Index (PSQI) and the Profile of Mood States (POMS) whilst consuming a standardised breakfast which we will provide you with.
- After a 10 minute break we will ask you to complete a cognitive function test battery which is a series of tests assessing your spatial awareness, verbal fluency, memory, executive function, attention and motor performance. All tests are completed using either a laptop or pen and pencil, and it should take no longer than 30 minutes to complete in total.
- You will then be asked to perform three maximal contractions of the first dorsal interosseus muscle (muscle in the hand that moves your index finger towards your thumb). While the rest of your hand remains still, we will ask you to push you index finger sideways against an immovable metal bar which measures that force that you produce (Figure 1).
- You will be asked to perform three maximal contraction of the quadriceps (thigh muscles) on a dynamometer (Figure 2).
- Finally, you will be asked to perform three countermovement jumps on a force plate where you will be asked to bend your knees to approximately 90 degrees and then immediately jump as high as possible, landing with both feet on the force plate at the same time.

Potential Benefits to You
- Experience as a participant in a Sports Science research trial
- Experience in a wide range of performance testing measures
- Free body composition analysis

Potential Risks to You
- There may be slight bruising from the blood sample
- Muscle soreness may be experienced during and in the days following the assessments

You are free to withdraw from testing at any time without explanation.

Contacts:

Mr Daniel Martin: 07773466063   Dr Kirsty Elliott-Sale

Daniel.Martin@ntu.ac.uk   Kirsty.Elliottsale@ntu.ac.uk

Appendix 15. Informed consent form used in Chapters 6 to 8

Subject Statement of Consent to Participate in the Investigation Entitled:

"The effect of menstrual cycle phase and oral contraceptive use on muscle function, cognitive function, energy expenditure and bone turnover"

1) I, _____agree to partake as a subject in the above study.

2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with *Daniel Martin* that this will involve me: *Attending the Sport Science laboratories at NTU Clifton Campus on 5 different occasions, with each visit lasting up to 2 hours. I will be required to provide blood samples, complete dietary and activity records, and undergo muscle function and cognitive function testing.*

3) It has also been explained to me by *Daniel Martin* that the risks and side effects which may result from my participation are as follows: *Some discomfort can occur through venepuncture although this will be carried out by trained members of staff.*

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent to participate in the study at any time and for any reason, without having to explain my withdrawal.

7) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.

8) I confirm that I have read the University's policy relating to the storage and subsequent destruction of sensitive information. I understand that sensitive information I have provided through my participation in this study, in the form of *menstrual history questionnaire, Pittsburgh sleep quality index and profile of mood states questionnaire* will be handled in accordance with this policy.

9) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

Subject signature: …………………………………………………………………
Date:_____

Independent witness signature: …………………………………………………
Date:_____

Primary Researcher signature: ………………………………………………….
Date:_____

Appendix 16. Participant information sheet used in Chapters 9 and 10

Participant Information Sheet

Project Title: The effects of acute energy restriction by diet or exercise on bone metabolism, muscle function and cognition.

We would like to invite you to volunteer in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. **One of the study researchers will go through the information sheet with you and answer any questions you may have.** Please feel free to talk to others about the study if you wish. You may take as much time as you require to decide whether you would like to participate. This information sheet tells you the purpose of this study and what will happen to you if you take part and gives you more detailed description about the conduct of the study. **Please ask if anything is not clear.**

Project description

Whilst many populations restrict their energy intake (i.e. athletes, military recruits, obese patients on a weight loss programme), recent research suggests that this may have negative consequences for bodily functions including the bone health, muscle function and cognition of these individuals. Although the unfavourable effects of energy restriction are becoming clearer, it is still not known if the method by which energy restriction is achieved (diet, exercise, or a combination of both) affects the response. Therefore, the current study aims to examine if there are different responses to energy restriction when it is achieved by dietary restriction or increased energy expenditure (exercise) for bone metabolism (physiological bone breakdown followed by new bone formation), muscle function and cognition.

What is the purpose of the study?

The study is part of a PhD thesis investigating the effects of energy restriction on bone metabolism, muscle function and cognition. The aim of the current study is to investigate the effects of 3 days energy restriction achieved by diet or exercise on bone metabolism, muscle function and cognition.

Why have I been invited?

We are looking for healthy individuals who perform regular moderate-to-high intensity exercise and who would be capable and willing to complete three experimental conditions:

1. 3 days controlled energy balance (~2000kcal per day)
2. 3 days dietary restriction (~600kcal per day)
3. 3 days increased energy expenditure (~2000kcal per day with ~1400kcal worth of exercise per day)

Do I have to take part?

It is entirely up to you to decide whether or not to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason.

What will happen to me if I take part?

You will be asked to complete preliminary assessment (P) to establish inclusion and exclusion criteria, take body composition measurements (DXA and ultrasound scan), undertake muscle and cognitive function tests and determine your fitness level (approximately 2 hours). Following this, you will be asked to record you habitual dietary intake and lifestyle physical activity for 3 days (H1-H3) before completing three experimental conditions. In each condition you will be asked to a member of the research team at the beginning of your next menstrual or oral contraceptive pill cycle (D1), undertake muscle function and cognitive function tests (D2 and D6) and undergo an

experimental period. In condition 1 you will consume an energy-balanced diet and perform no exercise. Condition 2 and 3 will include the same level of energy restriction; condition 2 will be achieved by manipulating diet only, whereas condition 3 will be achieved by increasing exercise energy expenditure only. During condition 3 you will be required to visit the laboratory to complete exercise sessions lasting approximately 2.5 hours on each experimental day (D3-5). Blood samples will be collected over the experimental period (10 minutes procedure) and analysed for the main outcomes. The three testing conditions will each be separated by approximately 25 days to allow recovery from blood sampling and to include three menstrual cycles for eumenorrheic (regularly menstruating) participants and three pill taking cycles for oral contraceptive users. The whole testing period will take approximately 14 weeks (see graph).

Expenses and Payments

You won't receive any payments for your participation in the study.

Food will be provided for the 3 experimental days (D3-5) in all 3 conditions.

What will I have to do?

During the preliminary assessment (P) we will explain the study to you, provide you the opportunity to ask questions and ask you for your written informed consent. You will then be asked to fill out questionnaires, undertake muscle cognitive function and muscle function tests, perform a fitness test and undergo body composition scans (this will be in the form of Dual-energy X-ray absorptiometry (DXA), which is used for making measurements of body composition and bone mineral density and an ultrasound scan used to measure the diameter of a muscle in your hand). For the DXA scan you will be asked to remove your shoes and any metal objects and lay on your back on the bed of the scanner. An x-ray beam will then pass slowly over your whole body for approximately 8 minutes. You will not feel any sensation of this beam. During the habitual assessment (H1-H3) you will be asked to record your habitual dietary intake and lifestyle physical activity. You will then be asked to complete three experimental conditions. In each condition you will be asked to notify the experimenter at the beginning of your next menstrual or oral contraceptive cycle (D1). On the next day (D2), you will be asked to visit the laboratory for a blood sample and to complete baseline muscle and cognitive function tests. The following 3 days of the protocol (D3-D5) will be the experimental period. Over the 3 experimental days you will undertake either condition 1, condition 2 or condition 3. In condition 1 you will consume an energy balanced diet and be asked to refrain from any exercise. In condition 2 you will be asked to refrain from any exercise and maintain a low-calorie diet for 3 days. In condition 3 you will provided with a normal diet and be asked to complete exercise sessions at 70% of your maximal oxygen uptake in two sessions per day (approximately 1 hour in the morning- 1 hour in the afternoon; each 1 hour will be performed in 15 minutes sessions with 5 minutes break between them) for 3 days. Similarly to D2, blood samples will be collected and muscle and cognitive performance tests will be conducted at the end of the protocol (D6). All blood samples will be taken from a vein in the forearm by an experienced phlebotomist.

What are the possible disadvantages and risks of taking part?

During the process of being scanned you will receive a small dose of radiation (30 µSv) which is very small compared to other X-ray procedures and is the equivalent to the additional cosmic radiation dose received from a flight from the UK to Spain. Exercise can result in injury and during the blood sample you may experience discomfort including mild pain, a sharp sensation or slight bruising. However, the assessments will be conducted under controlled conditions in facilities that are appropriate for each test in order to minimise the potential for injury. However, should you feel unwell or any pain during the testing then please stop immediately and inform the experimenter. There is always a risk for exercise to result in adverse cardiovascular events, however this is unlikely as the criteria for participation in this study stipulate that you must regularly exercise and will therefore be accustomed to exercising. You may experience minor side-effects of energy restriction including dizziness, headaches and difficulties in concentrating. You will be supervised at all times whilst in the laboratory and will be contacted by phone each morning and evening during the energy restriction conditions to confirm you are well. Detailed

instructions to seek medical advice in case of an emergency and/or to eat something if you feel unwell as a side-effect of energy restriction are provided.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study will contribute to the available knowledge concerning the effects of energy restriction on bone health, muscle function and cognitive function in physically active and obese/overweight populations. You will improve your understanding of your individual fitness as you will receive individual $VO_{2\,max}$ and heart rate results. Furthermore, you will be provided the results of your individual assessment of body composition (body fat percentage, lean body mass) and bone mass. Finally, if you decide to participate you will receive a full report indicating the results of your assessment of daily dietary intake.

What happens when the research study stops?

The information from the study (fitness test, body composition and bone mass measurement and dietary analysis) will be provided as feedback to you at the end of your participation.

What if relevant new information becomes available?

If new information comes available that is applicable to the safety of the study we will inform you of this information. If the study is stopped for any reason, you to be informed with regard to the reasons.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any point without providing a reason and without consequence.

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions using the contact details at in this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting Nottingham Trent University's technical manager, Mark Cosgrove Tel: **0115** **8486691**, who is independent of the research program and will take you through the complaints procedure.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. All information will be coded and stored securely. Any information about you which leaves the University will have your name and address removed so that you cannot be recognised (e.g. in case of a publication). All data will be used for analysis in the present study. All data will be destroyed no later than 5 years post the award of a PhD for the research students involved in this study.

Involvement of the General Practitioner/Family doctor (GP)

You may wish is seek advice from your GP, however we will not inform your GP.

What will happen if I don't want to carry on with the study?

If you withdraw from the study, we will ask you if you give us permission to use any data collected from you until that point. If you do not consent to this, we will delete all data pertaining to you that is stored on computers or hard copies and destroy any samples collected from you.

What will happen to any samples I give?

Your blood samples will be collected in coded tubes and will be stored in freezers contained within the Department of Sport Science laboratories. Your samples will only be identifiable to the research team. Your data from the body scan, dietary analysis, physical activity records and questionnaires will be stored in a locked cabinet or on a password protected university computer.

Your samples will be stored for no later than 5 years post the award of a PhD for the research students involved in this study

Your samples will be analysed within Nottingham Trent University and University of East Anglia on a collaborative basis.

What will happen to the results of the research study?

The results of the study will be provided as feedback to you and also published in a peer reviewed academic journal. Information will be provided as to the location of the publication when this information is known. You will not be identified in any report or publication.

Who is organising and funding the research?

The research is organised by Nottingham Trent University.

Who has reviewed the study?

The research is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by East Midlands NHS Research Ethics Committee.

Further information and contact details

All participants will be given an information form and signed consent form.

I'm unsure whether to participant or not?

Please take time to consider if you would like to participate. You may take as much time as you need to seek any advice as you see fit and decide whether you would like to participate. If you have any questions please don't hesitate to contact the research team on any of the methods listed below.

Contact Details:

Investigator:
Maria Papageorgiou, MSc
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Postgraduate Researcher
Biomedical, Life and Health Sciences Research Centre
School of Science and Technology,
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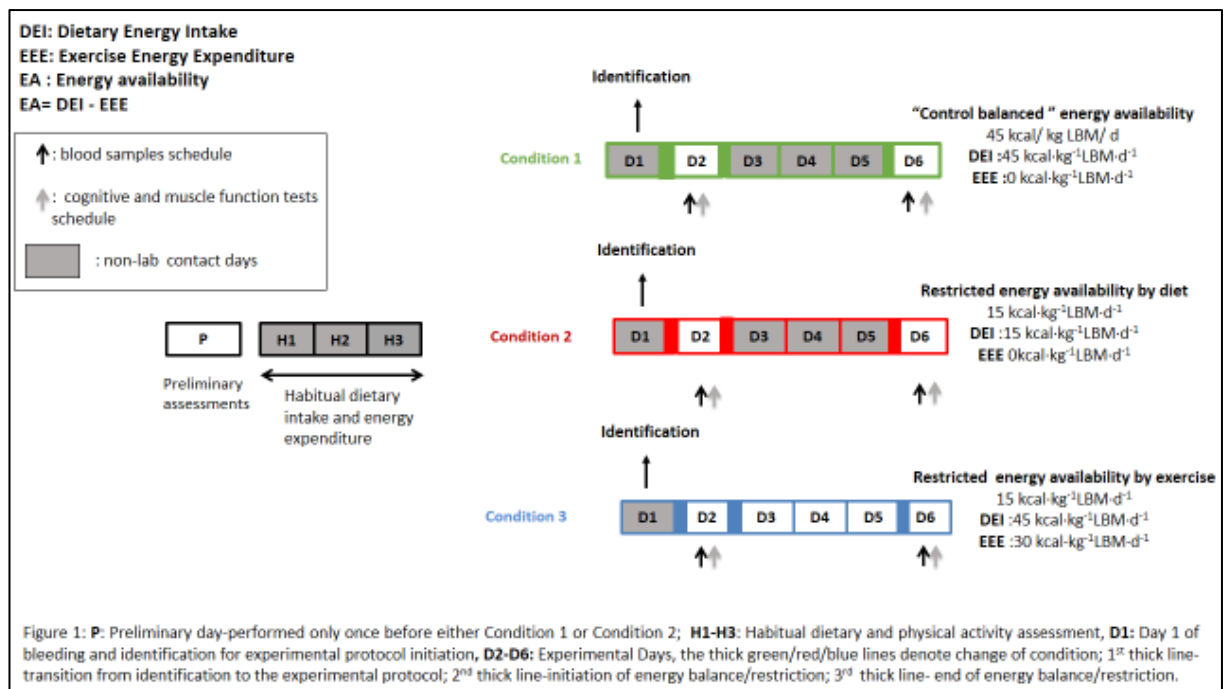Nottingham, UK.
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Figure 1: Outline of study design

Figure 2: Time commitment required by each experimental condition

Appendix 17. Informed consent form used in Chapters 9 and 10

INFORMED CONSENT FORM

Title of Project: "Effects of short-term energy restriction achieved by diet or exercise on bone turnover, muscle function and cognition"

Name of Researchers: Maria Papageorgiou and Dan Martin

1. I confirm that I have read the information sheet dated 10/10/2014 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand from the participant information sheet, which I have read in full, and from my discussion (s) with *Maria Papageorgiou and/or Dan Martin* that this will involve my participation in an energy restriction protocol for 3 days on two different occasions (exercise and diet) and energy balance for 3 days on one occasion. Moreover, this study will involve my participation in body composition measurements, muscle force production measurements, cognitive function tests and fasted blood samples (20ml on 6 occasions) during the study) ☐

3. It has also been explained to me by *Maria Papageorgiou and/or Dan Martin* that the risks and side effects which may result from my participation are as follows: slight bruising due to blood samples, muscle injury due to exercise sessions and side-effects of energy restriction. ☐

4. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

5. I understand that the information collected about me will be used to support other research in the future, and may be anonymously shared with other researchers. ☐

6. I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous. ☐

7. I understand that I will be consuming a multi-vitamin multi-mineral supplement. ☐

8. I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise, that would prevent me from partaking in this research. ☐

9. I agree to take part in the above study. ☐

_____   _____   _____
Name of Participant   Date   Signature

_____   _____   _____
Name of Person   Date   Signature
taking consent

Appendix 18. Published articles