The Cardiometabolic and Skeletal Profile of Female Endurance Athletes with Amenorrhea and Oligomenorrhea

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Abstract

Background

Menstrual disturbances, namely amenorrhea and oligomenorrhea are relatively common disorders in female endurance athletes as a result of low energy availability (LEA). LEA is characterised by the perturbation of several hormones of which are involved in the regulation of bone (re)modelling and also those with cardio-protective properties. The impact on bone health is relatively well understood, but the extent of cardiometabolic risk factors ranging along a scale of both time and severity of menstrual disturbances is yet to be determined.

Methods

In this observational study, 4 amenorrheic athletes (AA), 3 oligomenorrheic athletes (OA) and 5 eumenorrheic athletes (EA) completed the LEAF-Q and received measurements of stature, mass, resting heart rate, resting blood pressure, waist circumference, body composition using bioelectrical impedance analysis and dual energy X-ray absorptiometry (DXA). Bone Mineral Density was measured by DXA at the total body, total hip, femoral neck, and anterior-posterior lumbar spine (L1-L4).

Results

There were statistically significant differences in the total body and lumbar spine BMD Z-scores between amenorrheic and eumenorrheic athletes. Mean BMD Z-scores (-1.13 - 1.33) for the amenorrheic group were not outside of the normal range (>-0.2). Total body water (TBW) was at the top end of the normal range for the amenorrheic group, but not statistically significant from the eumenorrheic group. TBW was, negatively associated with waist:height ratio (R=-.874, p=<.001). Waist:height ratio was positively associated with total body BMD Z-score (R=.741, P=.006). BMD total body (Z-score) was positively associated with percentage body fat (PBF) (R=.682, p=0.015).

Conclusions

This study confirms the findings of previous work, that exemplify the differences in bone density between amenorrheic and eumenorrheic endurance athletes. Further studies need to be undertaken to confirm bone loss and better understand the time-course for any bone loss from onset of menstrual disturbance. Due to COVID-19 restrictions, the study sample size was limited and biochemical markers of cardiometabolic status were possible.

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Chapter 1 - Introduction

1.1 Background

Menstrual Disturbances in Female Athletes

Menstrual disturbances, namely amenorrhea and oligomenorrhea are relatively common disorders, and there has been difficulty determining the prevalence of each condition due to variability in type and its definition (DiPietro & Stachenfeld, 2006). Previously, the prevalence of amenorrhea in particular has been reported to be between 3.4% to 66% in some segments of the athletic population (Loucks & Horvath, 1985. Shangold, Rebar, Wentz, et al., 1990. Otis, 1992), compared with 2% to 5% in the general population. The much greater range between the athletic population is potentially reflective of how sport specific the prevalence of amenorrhea can be, especially given that there's such a tight range in the general population. Although amenorrhea can affect female athletes in any sport, there is often a higher prevalence reported in sports that are endurance based (Nattiv et al., 2007), and in sports that have a community of which emphasise leanness being of high importance, such as gymnastics and dance (Zanker et al., 2004).

In particular, in female athletes' functional hypothalamic amenorrhea (FHA) is the most common (Sonntag and Ludwig, 2012) and FHA is responsible for 20–35 % of secondary amenorrhea cases (Practice Committee of the American Society for Reproductive Medicine, 2006). This is reflective of altered levels of sex hormones and particularly common for those partaking in endurance sports, such as long-distance running, cycling, and swimming, the focus of this study. This is due to the energetically demanding nature of the exercise (Hutson et al., 2020) that can often cause female athletes to be at risk of being in an energy deficit. An energy deficit can lead to the development of the health and performance consequences of low energy availability (LEA) described in the relative energy deficiency in sport (RED-S) clinical model (Ackerman et al., 2019).

Low Energy Availability

LEA is defined as an energy deficiency relative to the balance between energy intake in the form of food, and energy expenditure required for activities of daily living, healthy bodily functions, growth, and exercise activities such as training and competition (Mountjoy et al,

2015). LEA is experienced by many athletes, with cross sectional studies highlighting that anywhere from 51% (Koehler et al., 2013) to 63% (Melin et al., 2014) of female endurance athletes suffer from low or reduced energy availability. LEA is characterised by the perturbation of several hormones of which are involved in the regulation of bone (re)modelling and also those with cardio-protective properties (De Souza & Williams, 2004). Therefore, the numerous athletes at risk of LEA and consequently of disrupting bone remodelling and exposing themselves to an unhealthy cardiometabolic profile suggests that this is a problematic area of sport that needs to be addressed. This is not only to aid sports performance but to ensure the optimum health of female athletes as a whole population. FHA is the most severe menstrual disturbance that LEA causes and is a reversible cause of ovarian disruption characterized by the absence of menses and chronic oestrogen deficiency (Alloway et al., 2016).

Relative Energy Deficiency in Sport (RED-S)

The International Olympic Committee (IOC) advise that early identification of LEA in athletes is crucial to prevent development of adverse health and performance outcomes, because LEA is the underlying aetiology of Relative Energy Deficiency in Sport (RED-S). The syndrome of RED-S is characterised by impaired physiological functioning caused by LEA and includes, but is not limited to, impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (Mountjoy et al, 2014). RED-S is an expansion of the female athlete triad, a model that links LEA, menstrual disturbances, and low bone mineral density (BMD) to one another and includes male athletes (De Souza et al., 2017). RED-S in female athletes is often characterised by irregular menstrual cycles, known as oligomenorrhea, or an absence of menstrual cycles, known as amenorrhea and low BMD. Alongside these health complications, an unfavourable lipid profile (Rickenlund et al., 2005) and lower resting glucose levels (Tornberg et al., 2017), of which are often associated with an increased risk to cardiovascular health, have been suggested to be consequences of RED-S.

Menstrual disturbances are most prevalent in sports emphasizing leanness and endurance sports such as running (Nattiv et al., 2007), where it may be as high as 50–65% (Melin et al., 2015. Dusek, 2001). Due to the high prevalence in endurance sports, this research thesis focuses on female athletes within this population, as previous research demonstrates that it is an at-risk group for RED-S.

Biological Pathways

A normal menstrual cycle depends on a successfully functioning hypothalamic-pituitary-ovarian (HPO) axis (Gordon et al., 2017), which is modulated by energy availability and exposure to stresses such as exercise. If the exercise carried out has an energy demand greater than the energy available, or if energy availability is low, the HPO axis is modulated by an increase in the activity of the hypothalamic-pituitary-ovarian axis (HPA) and subsequently suppresses gonadotrophin release (Mastorakos, 2008). Consequently, this affects the menstrual cycle and ovulation. This is because the hormonal synthesis, follicular development and increased luteal phase thermogenesis are energy-consuming processes (Harber, 2004) but aren't critical processes for survival. Therefore, when the body is in a state of low energy availability these processes are disrupted by the cessation of the HPO axis and an increase in the activity of the HPA axis, ceasing the release of gonadotrophins and so stopping the menstrual cycle, to reserve energy for more critical bodily processes (Koltun & De Souza et al., 2020).

A lack of oestrogen, termed hypoestrogenism, is consequently associated with the suppression of the HPO axis, as a result of LEA. Hypoestrogenism is due to the release of gonadotrophins, FSH and LH, being suppressed, resulting in the diminishment of oestrogen production and the reproductive system, to allow essential physiological mechanisms to continue (Gibbs et al, 2011). Oestrogen plays a key role in activating and stimulating bone remodelling and inhibits reabsorption of bone (Khosla et al., 2012). As such, hypoestrogenism compromises peak bone mineral accrual during adolescence and young adulthood and also the maintenance of BMD in later life. Ihle and Louckes (2004) demonstrated that bone formation is impaired within as little as 5 days of the onset of LEA in non-athletic women. Oestrogen has also been proven to have cardio-protective properties, and as a result, women with irregular menstrual cycles have been shown to have an increased risk for cardiovascular disease (CVD) (Solomon et al., 2013-2017).

1.2 Study Rationale

The risks associated with hypoestrogenism in amenorrheic athletes have been highlighted in many studies (Hutson et al., 2021). In elite female athletes with functional hypothalamic amenorrhea for at least 2 years, there was an inverse correlation between BMD and cardiovascular blood biomarkers which reported higher total cholesterol, apolipoprotein-A , and very-low-density lipoprotein when BMD was lower (Soleimany et al., 2012). This study highlighted both a relationship between and the decline of bone health and cardiovascular health, resulting from a lack of oestrogen and more broadly due to LEA.

Research Gaps

Evolving research demonstrates a continuous need for a better understanding of how menstrual disturbances resulting from LEA affects biological processes and leads to an overall decline in health and sports performance. There are key physical health concerns associated with amenorrhea and oligomenorrhea and there is a need for research to understand the altered hormonal profiles that consequently arise and the risks that they pose to both bone and cardiovascular health. In addition, research needs to be conducted in different athletic populations to ensure preventative measures can be taken for those in high-risk groups and that those in positions of leadership can understand prevention and early detection.

This research aims to investigate the altered physical profiles of athletes with menstrual disturbances when compared to eumenorrheic athletes, and how these changing profiles pose a risk to bone and cardiovascular health. It will focus specifically on endurance-based athletes, as keeping the study specific is more likely to produce reliable results reflective of a particular athletic population.

1.3 Research Aims

In this study the following research questions were addressed:

1. Are there differences between the cardiometabolic profile of female endurance athletes with and without menstrual disturbances?
2. Are there any differences between the skeletal profile of female endurance athletes with and without menstrual disturbances?

Answering these questions provided an insight into, and a better understanding of the cardiometabolic and skeletal profile of endurance-based athletes with menstrual disturbances, built off an existing knowledge of their associations with LEA and RED-S (Mountjoy et al., 2018).

Chapter 2. Literature Review

2.1 Menstrual Disturbances in Female Athletes

Reproductive function in women is dependent on the maintenance of metabolic homeostasis in each individual. This allows for an operational hypothalamic-pituitary-gonadal axis, which is the physiological function that results in a successful, undisrupted menstrual cycle. In situations where there is inadequate energy intake, termed low energy availability (LEA), to support all physiological functions, energy is repartitioned away from costly metabolically processes including growth and reproduction (Koltun & De Souza et al., 2020). This is in order to favour the most necessary processes for immediate organism survival, for example cellular maintenance, thermoregulation, and locomotion (Wade et al., 1992). As a result of energy repartitioning, the reproductive axis can be suppressed, leading to the development of menstrual disturbances. Further to this, it is evident that LEA plays a causal role on the induction of menstrual disturbances (Williams et al., 2001).

LEA has many consequences, but one of the most common in female athletes is menstrual disturbances, namely amenorrhea and oligomenorrhea. However, menstrual disturbances can range in their degree of severity, often from subclinical luteal phase defects, which often go undiagnosed, through to oligomenorrhea and amenorrhea (Nattiv et al., 2007) at the end of the continuum. De Souza et al. (2009) estimated that approximately 50 % of women who exercise regularly experience subtle menstrual disorders and approximately 30 % of women have amenorrhea.

It should be noted though, that even oligomenorrhea and amenorrhea operate on their own separate continuums and also range in degree of severity, often determined by their duration and additional symptoms/side effects. Athletes who do not experience menstrual disturbances are known as eumenorrheic, which is defined as a normal menstrual cycle at an interval that is near the median for young adult women. In young adult women, menstrual cycles recur at a median interval of 28 d that varies with a standard deviation of 7 d (Nattiv et al., 2007). Menstrual disorders in athletes are associated with broader diagnoses', falling under the Female Athlete Triad (FAT) and Relative Energy Deficiency in Sport (RED-S) as an element of each of these clinical entities. These entities only include severe menstrual

disturbances, defined as amenorrhea and oligomenorrhea. However, irrelevant of their severity, all menstrual disturbances present a clinical concern, due to their association with a hypoestrogenic state, which contributes to longer term health complications both for the cardiovascular system (O'Donnell and De Souza, 2004) and bone health (Prior et al., 1990). Notably though, these menstrual disturbances do exist along a continuum, ranging from mild and moderate subclinical concerns to severe clinical outcomes (De Souza, Koltun & Williams, 2019).

2.1.1 Amenorrhea

Amenorrhea is the 'absence of menstrual bleeding' and can be classified as either primary or secondary. In relation to the 2014 International Olympic Committee's consensus statement, primary amenorrhea is defined as not having menarche, which is the first menstrual bleeding, by the age of 15 (American Society of Reproductive Medicine Practice Committee, 2008). Whereas, secondary amenorrhea is defined as established menstrual cycles with an absence of menstrual bleeding for 6 months or for a length of time equivalent to a total of at least 3 of her previous cycle lengths (West, 1998. Redman and Loucks, 2005).

Amenorrhea is a relatively common disorder, and there has been difficulty determining its prevalence due to variability in type and its definition. Previously amenorrhea has been reported to be between 3.4% to 66% in some segments of the athletic population (Loucks & Horvath, 1985. Shangold, Rebar, Wentz, et al., 1990. Otis, 1992), compared with 2% to 5% in the general population. The much greater range between the athletic population is potentially reflective of how sport specific the prevalence of amenorrhea can be, especially given that there's such a tight range in the general population. Updated individual prevalence estimates of primary and secondary amenorrhea in 13 (n=2216) and 34 studies (n=5607), respectively, ranged from 0%– 56.0% and 1%–60.0% (Gibbs, Williams & De Souza, 2013). Even updated data highlights a broad range within the athletic population as did the previous estimates, suggesting that even over a 20-30 year period, amenorrhea remains a highly fluctuating issue dependent on the sporting population researched (Lania et al., 2019).

It has been highlighted by many studies over a sustained period of time, that amenorrhea is more prevalent in sports that emphasise and encourage leanness (Carlberg et al., 1983. Torstveit and Sundgot-Borgen, 2005. Nattiv et al., 2007. De Souza et al., 2014), such as

endurance and aesthetic based sports (Table 1). Athletes competing in sports that emphasise leanness are also at a higher risk of developing disordered eating patterns and a decline in athletic performance (Krentz and Warschburger, 2011). These categories are further examined in Table 2, with endurance-based sports including middle and long distance running as well as cycling. Likewise, aesthetic-based sports include gymnastics and dance, but distance running can also fall under this category. The estimated prevalence of amenorrhea amongst distance runners is as high as 60% (Pollock et al., 2010), highlighting a necessary need for further understanding into the physiological and psychological impacts of these disorders.

Table 1: Proportion of Female Athletes with Menstrual Disturbances by Sport Category. (Adapted from data collected from a Norwegian population, by Torstveit and Sundgot-Borgen, 2005).

Sports Category	N	% With Menstrual Disturbance
Technical	7	13.5
Endurance	59	30.5
Aesthetic	20	30
Weight-Class	17	5.9
Ball-Game	158	12.7
Power	19	15.8
Anti-gravitational	9	11.1

Table 2: The Estimated Prevalence of Low Energy Availability (LEA), Amenorrhea and Bone Mineral Density (BMD) in entire Female Athlete Populations, per Endurance based or Aesthetic based sport.

Category of Sport	Type of Sport	Estimated prevalence of LEA in population	Estimated prevalence of amenorrhea in the population	Estimated prevalence of low BMD
Endurance	Middle/Long Distance Running	18–58% sub-elite/elite middle-distance female and male athletes (Melin et al., 2019) *best estimate found	60% (Pollock et al., 2010)	40-45% (Melin et al., 2015; Pollock et al., 2010; Tam et al., 2018)
	Cycling	Estimated at 70-90% (Viner et al., 2015), but a small sample size (10) was used.	Not been determined	Not been determined in women
Aesthetic	Dancers	57% (N, Keay et al., 2020)	51% of professional dancers 34% of amateur dancers (Bacchi et al., 2013) 44% in ballet	23-40% of the lumbar spine
	Gymnastics	44.8% (Silva and Paiva, 2015)	Primary amenorrhea - 15–20% of elite female gymnasts (Helge and Kanstrup, 2002). Secondary amenorrhea - 40–60% of this population (Zanker et al., 2004)	Despite primary or secondary amenorrhea, late adolescent and young adult gymnasts were shown to have greater BMD than normally active females (Helge and Kanstrup, 2002) or females engaged in less osteogenic activities such as running (Robinson et al., 1995)

Functional Hypothalamic Amenorrhea

Functional Hypothalamic Amenorrhea (FHA) is one of the most common causes of secondary amenorrhea and is responsible for 20–35 % of secondary amenorrhea cases. However, FHA is only responsible for approximately 3% of primary amenorrhea cases (Practice Committee of the American Society for Reproductive Medicine Current evaluation of amenorrhea, 2006). Amenorrheic athletes most often experience FHA as their type of amenorrhea, as low energy availability affects the output of the hypothalamic gonadotropin-releasing hormone (Sonntag and Ludwig, 2012), which is what subsequently alters the menstrual cycle in these circumstances.

2.1.2 Oligomenorrhea

Oligomenorrhea which, alongside amenorrhea, is associated with the FAT and RED-S, is specifically defined as the presence of irregular and inconsistent menstrual cycle intervals greater than 45 days (Mountjoy et al., 2014). This is defined through the exclusion of the presence of hyperandrogenic profiles, where the body produces excess testosterone and disrupts the menstrual cycle (Rosenfield & Ehrmann, 2016). Oligomenorrheic cycles can present with or without an ovulatory event (De Souza et al., 2010), therefore arguably making this condition more difficult to identify. The estimated range in prevalence of oligomenorrhea in athletic populations was 3.5 %–52.5 % (23 studies: n=4044) (Gibbs, Williams & De Souza, 2013). Similarly, to amenorrhea, this encompasses a broad range, suggesting that menstrual disturbances as a whole are specific to sporting populations. Additionally, the range for oligomenorrhea may be so broad as inconsistent menstrual cycles, as opposed to amenorrhea, are harder to define and consistently track in individual athletes.

It is evident that menstrual disturbances have always been a pressing issue among female athletes, shown through the extent of past research studies exemplifying this. Prior to the formation of RED-S, many studies evaluating the full female athlete triad among competitive athletes, all of whom were representing a variety of sports, highlighted that the prevalence of oligomenorrhea or amenorrhea ranged from 18.8%–54% (Hoch et al, 2009. Nichols et al, 2006). This demonstrates that menstrual disturbances within the athletic population have

always been present, and that there is a continual need to further the understanding of the homeostatic disruption low energy availability causes.

2.1.3 Eumenorrhea

Eumenorrhea is defined as menstrual cycles at intervals near the median interval for young adult women. In young adult women, menstrual cycles recur at a median interval of 28 d that varies with a standard deviation of 7d (Nattiv et al., 2007), therefore regular cycles occur at intervals between 21 and 35 days. However, in adolescents, the cycles range between 21 and 45 days (ACOG committee, 2006). Therefore, the spectrum of menstrual disturbances ranges from Functional Hypothalamic Amenorrhea at the more severe end to eumenorrhea at the optimal end, and this is shown in Figure 1. Although a more simplified diagram of a much more complex entity now, it explicitly indicates that optimal energy availability promotes bone health and development indirectly by preserving eumenorrhea and oestrogen production that restrains bone resorption, and directly stimulating the production of hormones that promote bone formation. As a result, BMD is often above average for the healthy athlete's age (Nattiv et al., 2007). This diagram emphasises simplistically the importance of athletes being eumenorrheic and having a normally functioning menstrual cycle in order to promote optimal bone health and reduce the risk of injury from fractures, to allow for peak performance. Therefore, amenorrhea is not a condition that should be considered 'normal' or acceptable in female athletes (Rosen, 2018) as it hinders both long term and short-term success.

Figure 1: Changes in energy availability and the hormonal profile of female athletes across a continuum (Taken from De Souza et al., Current Status of the Female Athlete Triad: Update and Future Directions 2017, volume 15, p577–587).

Despite both the clinical entities of RED-S and the female athlete triad ranging in level of severity on a spectrum similarly to what is shown in Figure 1, with amenorrhea being at the extreme end, it has been proven that this continuum is relative to the individual. The individuality of this continuum is evident in studies by Loucks and Horvath (1984) who confirmed that there is no specific body fat percentage below which regular menses ceases. Some athletes with amenorrhea regain their menses after intervals of rest, even without an increase in body mass or body fat, suggesting that amenorrhea is not caused solely by low body mass or body fat (Torstveit and Sundgot-Borgen, 2003). Athletes who appear to be at the greatest risk of developing oligomenorrhea and amenorrhea do share some common factors, such as beginning training at an early age (prior to the age that normal menarche starts, 12 to 16 years of age), adhering to intense training regimens (Snow-Harter, 1994) but most importantly have low energy availability as seen in Figure 1.

2.1.4 Luteal Phase Defects

The luteal phase is one stage of your menstrual cycle, and it occurs after ovulation and around 12-14 days before menses. During this time, the lining of the uterus thickens to prepare for a possible pregnancy. Therefore, luteal phase defects lead to the improper growth of the lining due to a lack of progesterone (Reed & Carr, 2015).

Luteal Phase defect can often go unnoticed in athletes, as it does not tend to result in a lack of menses. Therefore, athletes who appear to be eumenorrheic often have their menstrual disturbance going undiagnosed. A study conducted by Loucks and Thuma (2003) highlighted that disruptions in the pulsatility of the luteinising hormone can be seen after only 5 days of reduction in energy availability (EA) to 30 kcal/kg FFM per day, highlighting that menstrual disturbances such as luteal phase defects can be brought about in a short period of time, reinforcing the continuum that they range across.

These findings have been extended more recently in a study carried out by Koltun et al., (2020) which highlighted that reducing EA by 10 units from 38 kcal/kgFFM/d to 28 kcal/kgFFM/d over 3 to 4 menstrual cycles, via an intervention similar to that which may be used by women trying to lose weight, is sufficient to significantly reduce LH pulse frequency and increase the likelihood of developing luteal phase defects and anovulation. Therefore,

low energy availability is the catalyst that causes menstrual disturbances ranging from luteal phase defects to functional hypothalamic amenorrhea.

2.2 Causes of Menstrual Disturbances in Female Athletes

2.2.1 The Energy Availability Threshold

Energy availability (EA) is defined as the daily dietary energy intake minus the daily exercise energy expenditure corrected for fat-free mass (FFM) (Loucks, 2004). The spectrum of EA ranges from optimal EA to LEA, with or without the presence of disordered eating (Nattiv et al., 2007). This means that athletes who do not struggle with an eating disorder, but do not consume enough dietary energy in relation to the extent of their exercise, can still have low energy availability and menstrual disturbances too. Rigorously controlled laboratory trials in women have shown that the optimal EA for healthy physiological function is typically achieved at an EA of 45 kcal/kg FFM/day (188kJ/ kg FFM/day) (Loucks and Heath, 1994. Loucks and Thuma, 2003). More recently, it has become clear that many athletes affected by the FAT and RED-S do not exhibit pathologic eating behaviours, and so, as previously mentioned, their LEA is unintentional (Melin et al., 2015).

It is noted that an EA of 30 kcal/kg/ FFM roughly equates to the average resting metabolic rate (RMR), and so EA <30 kcal/kg FFM per day affects bone remodelling and disrupts menstrual function and bone mineralization (Loucks and Thuma, 2011). Many systems are substantially perturbed at an EA <30kcal/kg FFM/day (125kJ/kg FFM/day), making it historically a targeted threshold for LEA (Mountjoy et al., 2018). As LEA has proven to be successful in explaining markers of suboptimal health and function in both laboratory (Loucks & Heath, 1994. Loucks & Thuma, 2003) and field settings (Melin et al., 2014. Vanheest et al., 2014), an assessment of energy availability could serve as a tool for diagnosis as well as in prevention and management of RED-S or the FAT (Mountjoy 2018). However, recent evidence suggests that this cut-off of below 30 kcal/kg FFM/day does not predict amenorrhea in all women (Lieberman et al., 2018; Reed et al., 2015) and that further research is needed to understand menstrual disturbances on the less extreme end of the spectrum, such as luteal phase defects and oligomenorrhea, before an athlete becomes amenorrheic.

Further to this, the correlation between EA and menstrual disturbances has also been demonstrated in non-athletes, in a study by Reed et al. (2015), EA was reduced via manipulation of energy intake and exercise energy expenditure over several menstrual cycles in untrained, previously eumenorrheic subjects. The study found that the frequency of menstrual disturbances (including luteal phase defects, anovulation, and oligomenorrhea) were affected by the magnitude of energy deficit compared to baseline needs, again reinforcing that energy availability is displayed along a continuum with differing menstrual disturbances. However, a specific threshold of EA below which menstrual disturbances occurred was not identified, suggesting it is unique to the individual. This continuum differs in each individual and further understanding is needed in order to diagnose menstrual disturbances faster, whilst also investigating their impacts on other parts of the body. Likewise, although there is clear evidence of the correlation between EA and menstrual disturbances, there is still a lack of understanding for the interplay of change in short-term and long-term EA and more subtle menstrual disruption.

Energy Availability in Eumenorrheic Athletes

A study by Reed et al. (2015) involved a cross-sectional analysis of EA. This was measured using 3-day diet logs to determine energy intake and a combination of exercise logs and heart rate monitoring to measure estimated exercise energy expenditure in female athletes with eumenorrhea and various menstrual disturbances. They reported that the mean EA was >30.0kcal/kg FFM/day in all the groups and EA did not discriminate subclinical forms of menstrual disturbance. However, EA was lower in amenorrheic athletes compared with eumenorrheic athletes (mean 30.9 vs 36.9 kcal/kg FFM/day). Therefore, although Eumenorrheic athletes will have a significantly higher EA than their amenorrheic counterparts, there is not necessarily a defined threshold consistent for every individual to comply to, in terms of what classifies each person as having optimal or low EA.

Low Energy Availability

Low energy availability (LEA) is defined as inadequate energy intake relative to exercise energy expenditure, and it is the main factor triggering the unfavourable health and performance consequences associated with RED-S (Mountjoy et al., 2018). Since the

publication of the International Olympic Committee (IOC) consensus papers on RED-S in 2014 and 2018 (Mountjoy et al.), scientific evidence for the risk of and performance consequences of LEA has grown. The prevalence of LEA in various sporting populations is estimated to range from 22% to 58% (Logue et al., 2020). However, an accurate estimation of the prevalence of LEA remains problematic due to continuing variability in the methods used to estimate EA (Logue et al, 2018).

2.2.2 Pathological causes of amenorrhea

There are many causes pathological and physiological causes of both primary and secondary amenorrhea, as a result of both internal and external factors dependent upon the individual. Pathophysiological types of amenorrhea include FHA, often associated with LEA, and therefore externally contributing factors. On the other hand, amenorrhea can be caused by diseases, stemming from genetic and environmental factors, or a combination of both. For example, Polycystic Ovarian Syndrome (PCOS), which results from an imbalance of female sex hormones and leads to cysts, containing an egg that would be used for fertilisation, on the ovarian antral follicles. This cyst prevents ovulation and disrupts the menstrual cycle, resulting in amenorrhea. Although both types of amenorrhea are caused by different initial factors, amenorrhea in both cases results from disrupted secretion of the pulsatile gonadotropin-releasing hormone (GnRH) from the hypothalamus (Golden and Carlson et al., 2008).

Pathophysiology of Functional Hypothalamic Amenorrhea

As previously highlighted, Functional Hypothalamic Amenorrhea (FHA) is a type of secondary amenorrhea accounting for around 30% of cases in women of reproductive age, and the most common in female athletes. It is a form of menstrual dysfunction which results from LEA and therefore leads to hormonal changes likely occurring to conserve energy for more important bodily functions or to use the body's energy reserves for vital processes (De Souza et al., 2020). LEA alters levels of metabolic hormones and substrates, for example, insulin, cortisol, growth hormone, insulin-like growth factor-I (IGF-I), 3,3,5-triiodothyronine, ghrelin, leptin, peptide tyrosine– tyrosine, glucose, fatty acids, and ketones (Wade and Jones, 2004), disrupting homeostasis in the body.

Both metabolic challenges and psychological stressors are implicated in the pathogenesis of FHA (Genazzani et al., 1995). FHA is characterized by the absence of menses and profound hypoestrogenism due to suppression of the hypothalamic-pituitary-ovarian (HPO) axis by the aberration of GnRH secretion (Sonntag and Ludwig, 2012). There are three main types of FHA, all of which are interrelated: stress-related amenorrhea, weight loss-related amenorrhea, and exercise-associated amenorrhea (Meczekalski et al., 2008). Therefore, the causative agents; undernutrition, weight loss, excessive exercise or a combination of all factors are what cause LEA and result in the metabolic imbalance associated with the development of FHA. The imbalances seen are a form of adaptation in order to reduce total energy expenditure, whereby the body attempts to obtain a new energy balance and steady state (Loucks, 2013).

As exercise carries an energetic cost it may therefore act as a metabolic stressor, and so an athlete may have a stable BMI and not excessively low in body mass or fat levels yet have impaired physiological function due to LEA (Drew et al., 2017. Loucks, 2013). The causative agents of menstrual disturbances are multifactorial, as there are many neuro-modulatory signals that alter hypothalamic GnRH function, without the athlete having to necessarily fall below a certain threshold definition. These agents include both inhibitory and stimulatory inputs, aligning GnRH function with the internal and external milieu, or environment, of the individual (Navarro and Kaiser, 2013). An overview of each of the biological pathways affected by LEA can be seen in Figure 2, exemplifying the multifactorial nature of menstrual disturbances.

The key mechanism that is disrupted and results in FHA is the suppression of the gonadotropin releasing hormone (GnRH) in the hypothalamic-pituitary-ovarian axis. This results in low follicle stimulating hormone (FSH) and luteinizing hormone (LH) being released from the anterior pituitary. Due to the lack of these hormones, the feedback mechanism here is disrupted and so the ovarian granulosa cells do not receive a signal to produce oestradiol, one of the three forms of oestrogen (Shufelt, Torbati & Dutra, 2017).

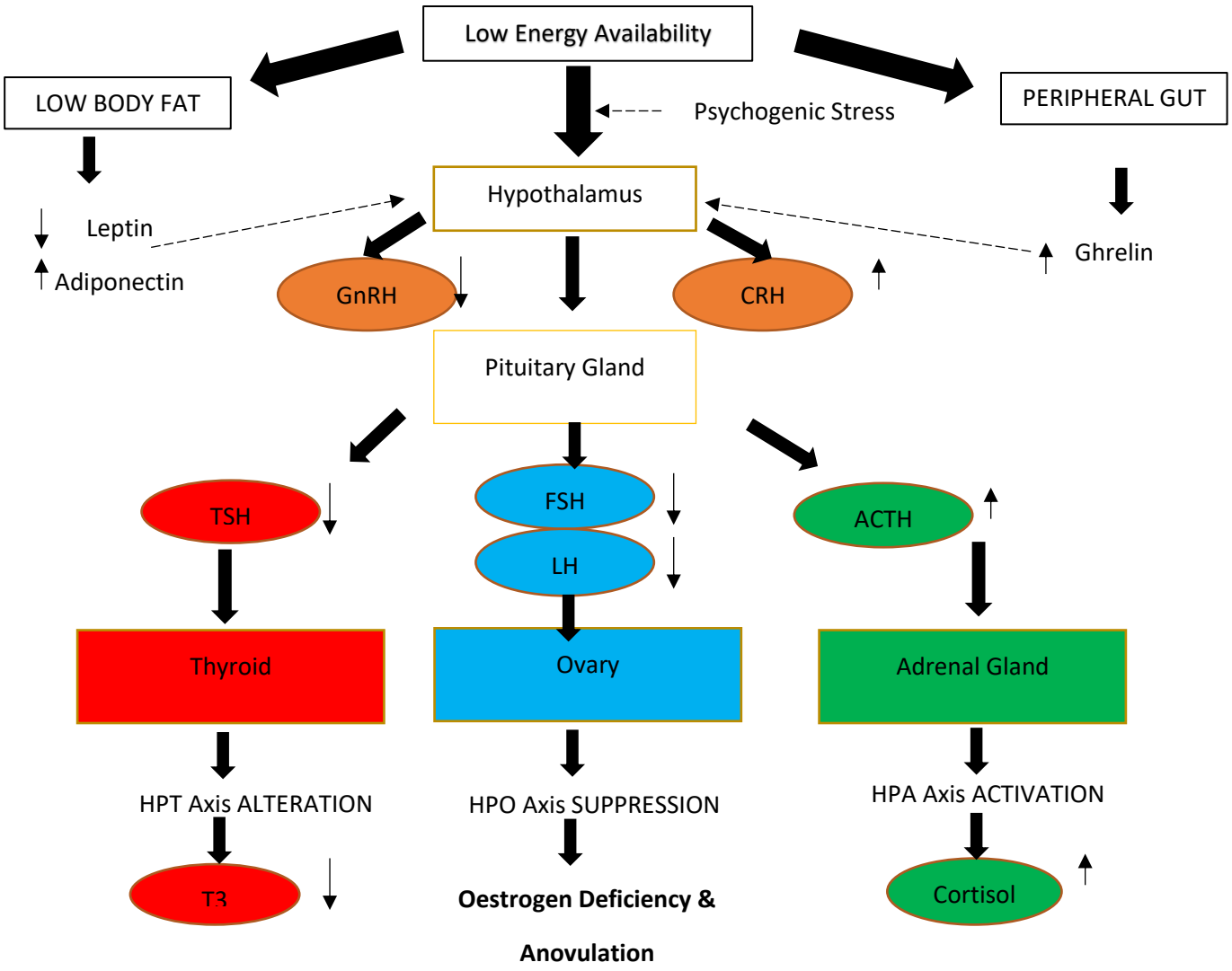

Figure 2 : The different biological pathways affected by LEA, their outcomes and how they lead to oestrogen deficiency and therefore menstrual disturbances (Adapted from O'Donnell, Goodman & Harvey, Cardiovascular Consequences of Ovarian Disruption: A Focus on Functional Hypothalamic Amenorrhea in Physically Active Women 2011, volume 96(12), p 3638–3648).

2.2.3 Axis Changes

The Hypothalamic-Pituitary-Ovarian Axis (Figure 2 - blue)

FHA is caused by the disruption of the hypothalamic-pituitary-ovarian (HPO) axis, a tightly regulated system controlling female reproduction that allows for the cyclic production of gonadotropic and steroid hormones and is essential for reproductive health. This cycle is tightly regulated to select a dominant follicle for ovulation, meanwhile priming the

endometrium for implantation (Mikhael et al., 2019). This complex regulation can be negatively impacted when pathologies occur within any juncture of the HPO axis.

LEA results in the suppression of the HPO axis due to the disruption of the leptin-controlled pathway (Figure 2) (Laughlin and Yen, 1997. Cunningham et al., 1999. Hilton and Loucks, 2000). Leptin is the product of an obesity gene, and it is produced in several organs (Hama et al., 2004). Leptin's main physiological role is to regulate hunger and satiety (Dhillon and Belsham, 2011), essentially controlling appetite and responding to energy availability (Chan and Mantzoros, 2005), also acts centrally to influence reproduction. Leptin's effects are only exerted over a narrow range of concentrations (Caprio et al., 2001), and so responds to a negative energy balance, meaning that menstruation is not possible if leptin levels drop below a critical level (Korsten-Rech, 2011). Many studies have confirmed that the energy balance is more negative and overall energy availability lower in adult athletes with menstrual disturbances compared to eumenorrheic controls (Morghental, 2002).

This is due to the fact that leptin's plasma level is directly related to total body fat stores, as Leptin increases after a couple of days of overfeeding and levels decreases within hours with the onset of hunger. The changes in leptin concentration are primarily regulated by insulin, cortisol, and reproductive hormones. Leptin acts in hypothalamic centres to modulate long term responses the body's declining energy state. Therefore, when the body starts to move into a negative energy imbalance, away from the usual positioning, leptin levels decrease, and the response to this is a reduction in oestradiol, which subsequently leads to menstrual disturbances (Yenilmez, 2020).

Functional Hypothalamic Amenorrhea (FHA) is classified as hypogonadotropic hypogonadism related to an aberration of the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Gordon, 2010). Lower energy availability and therefore lower concentration of leptin results in the suppression of the pulsatile secretion hypothalamic gonadotrophin-releasing hormone (GnRH) (Marquez and Molinero, 2013). Therefore, this deficient secretion of the GnRH occurs as a result of low energy availability, disrupting a leptin-controlled pathway, as the presence of Leptin stimulates the release of gonadotropins from the hypothalamus, by stimulating GnRH pulsatility (Figure 3) (Carro et al., 1997).

Subsequently, this deficient secretion of GnRH leads to a reduced secretion of the gonadotrophins' named luteinising hormone (LH) and follicle stimulating hormone (FSH), as seen in Figure 3, which are usually secreted by the pituitary gland secondary to pulsatile stimulation by hypothalamic GnRH.

As result, the lack of circulating gonadotrophins then prevents full folliculogenesis (Hamm et al., 2004) and ovulatory ovarian function and stimulation (Spicer and Francisco, 1997) and causes a fall in the levels of oestradiol, an oestrogen steroid hormone (Figure 3), and progesterone (Meczekalski et al., 2008). It has been demonstrated that providing exogenous GnRH or gonadotropins restores folliculogenesis and therefore provides evidence for the involvement of this disrupted pathway (Knobil and Plant, 1978).

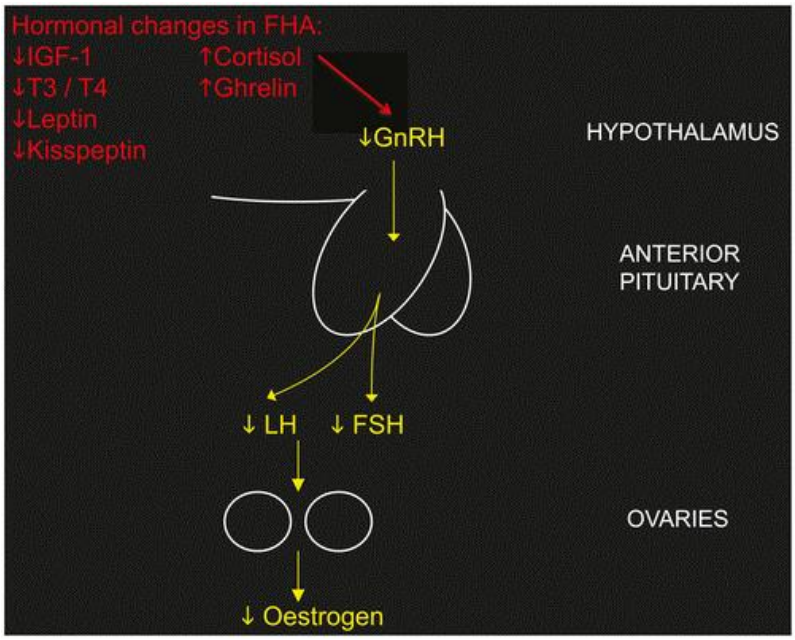

Figure 3: The effect of hormonal abnormalities associated with FHA on suppressing the hypothalamic–pituitary–ovarian axis (From Roberts et al., Current understanding of hypothalamic amenorrhoea 2020, volume 11, p 2042018820945854.)

FHA, functional hypothalamic amenorrhoea; FSH, follicle-stimulating hormone; GnRH, gonadotrophin-releasing hormone; IGF-1, insulin-like growth factor 1; LH, luteinising hormone; T3, triiodothyronine;T4,thyroxine.

The Hypothalamic-Pituitary-Adrenal Axis (Figure 2 – green)

The HPO axis and the hypothalamic–pituitary–adrenal (HPA) axis are tightly linked (Figure 2), with the HPA axis activated by nutritional or other psychological stress, reducing GnRH secretion and subsequent LH pulsatility from the pituitary gland (Mastorakos, 2008). A typical feature of FHA is hypothalamic–pituitary–adrenal axis activation, related to stressing factors, and this is believed to be one of the important pathogenetic factors in FHA patients (Meczekalski et al., 2008). A reduction in GnRH drive is closely linked with the activation of the HPA axis in both amenorrheic athletes and non-athletes. This link is because, when a form of stress activates the HPA axis, this in turn increases corticotrophin-releasing hormone (CRH) secretion and sequentially (Figure 2), increased secretion of adreno-corticotrophin from the pituitary and cortisol from the adrenal gland (Loucks et al., 2001).

The administration of CRH has been shown to inhibit gonadotrophin release in both healthy female volunteers (Barbarino et al., 1989) and monkeys (Xiao et al., 1989), yet conversely administration of a CRH antagonist stimulates release of GnRH (Nikolarakis et al., 1988). Furthermore, it has been shown that seemingly minor stressors in monkeys, that alone would have minimal impact on reproductive function, can interact synergistically. As a result, the combinations of stressors cause a greater impairment of the reproductive axis than any single stressor alone (Williams et al., 2007). The hypothalamic–pituitary disturbances in FHA are deemed to be on a spectrum, as are the Female Athlete Triad and RED-S; the clinical entities encompassing FHA. They can be very broad and include a lower mean frequency of LH pulses, the complete absence of LH pulsatility, as well as a normal-appearing secretion pattern and higher mean frequency of LH pulses (Genazzani, 2005).

Notably, the mechanistic link between LEA and menstrual disturbances is proposed to act through a slowing of LH pulse frequency, which is a proxy indicator of decreased gonadotropin-releasing hormone pulsatility (Berga et al., 1989). Initial cross-sectional investigations observed conflicting results and report low (Ronkainen, 1985), high (Schwartz, 1981) and similar (Yahiro, 1987) basal LH concentrations in oligomenorrheic athletes compared with healthy women. However, when examining the pulsatile secretion of LH, exercising women with menstrual disturbances displayed slower LH pulse frequency compared with eumenorrheic athletes and with sedentary women with regular menstrual

cycles (Loucks et al., 1989. Pirke et al., 1990). This highlights how LH pulsatility, alongside menstrual disturbances, acts along a spectrum of differing severity, unique to each individual. However, it in the longer term, it still remains unclear how reduced EA over a sustained duration, for example over years, relates to LH pulsatility and how a slowing of LH pulsatility translates to alterations in menstrual cycle quality (De Souza et al., 2019). Therefore, the long-term effects of EA and how that impacts the location of menstrual disturbances on a spectrum over a given time is not well understood.

The Hypothalamic-Pituitary Thyroid Axis (Figure 2 – blue)

T3 is the most active form of thyroid hormone and physiological processes, such as growth, metabolism, body temperature, and heart rate, are regulated by T_3. In addition, resting energy expenditure (REE), total daily energy expenditure and oxygen consumption are also tightly coupled with measures of total T_3 (Danforth & Burger, 1989). There are multiple early studies from animal and human experiments that demonstrates how general energy and macronutrient intake directly influence on thyroid hormone status and indirectly on REE (Burger et al., 1980., Rosenbaum et al., 2000., Wimpfheimer et al., 1979).

Measurements of total T_3 are commonly used to indicate the presence of an energy deficiency, as it is suggested that reductions in serum total T_3 concentrations initiate energy conservation mechanisms, in order to restore homeostasis (Burger et al., 1980., Wimpfheimer et al., 1979). Studies of sedentary, regularly menstruating women demonstrated the effect of manipulating EA on total T3. This was done through both increased exercise energy expenditure and decreased energy (caloric) intake. In response to the induction of a low EA, reductions in total T_3 were observed. Subsequently this was prevented when individuals were given extra calories to compensate for the induced energy deficiency (Loucks et al., 1993). Therefore, total T_3 is a biomarker sensitive to changes in EA, and hence an indicator of LEA, whether this is associated with changes in energy restriction and/or changes in exercise training in women (Loucks et al., 1993., Loucks & Heath, 1994).

A number of studies have highlighted that there are causal links between reproductive function and energy status, indicated through T3 levels. The induction and reversal of amenorrhea in female monkeys was correlated with changes in circulating total T3, with a

significant 18% increase in T3 levels observed during resumption of regular menses (14). Additionally, in humans, low concentrations of total T3 have been linked to reproductive dysfunction in exercising women with amenorrhea (Berga et al. 1989., De Souza et al., 2007., Loucks et al., 1992). For example, Loucks et al. (1992) demonstrated that amenorrheic female athletes had suppressed total T3 concentrations compared to their eumenorrheic counterparts.

In reference to menstrual disturbances acting along a continuum, changes in total T3 are present in a dose response manner, such that increases in T3 (as a form of energy conservation) are observed with increasing severity of menstrual disturbances (De Souza et al., 2007). These studies suggest that menstrual dysfunction is linked to energy conservation mechanisms, as low T3 levels are observed when an underlying energy deficiency is present and the regaining of successful menstrual function is associated with the restoration of an adequate energy intake relative to energy expenditure (Williams et al., 2001, Williams et al., 2001), which can be observed by increases in total T3 (Allaway, Southmayd & De Souza, 2016).

Hypoestrogenism

The term for FHA was originally called hypothalamic hypoestrogenism, as the final endocrinological consequence of impairment in GnRH and gonadotropin pulsatile secretion is profound hypoestrogenism. The cause of hypoestrogenism is due to decreased LH and FSH secretion, which results in a deficiency of endogenous oestrogen (Grossman-Rimon, 2019). The hypoestrogenic status has a negative influence on different aspects of female health, not only in menopausal women but also in young individuals (Gordon, 2010. Meczekalski et al., 2008). Particularly in young women, normoestrogenism and metabolic homeostasis have a critical significance for normal bone metabolism, the cardiovascular system and mental health (Meczekalski et al., 2014). Therefore, prolonged hypoestrogenism which occurs in young women may have important consequences on women's future health. The effects of LEA on reproductive hormones and menstrual function in female athletes have been well described (Loucks, Verdun & Heath, 1998; Tornberg et al., 2017) although the complex hormonal signalling pathways underpinning these effects are still being fully elucidated.

2.2.4 Additional Common Hormonal Abnormalities

Peripheral hormones such as leptin, adiponectin, ghrelin, PYY, and cortisol cross the blood-brain barrier and exert varying effects on regulatory mechanisms within the hypothalamus. These hormones also control activation and inhibition of appetite through neuronal activity in the ARC, a key nucleus of the hypothalamus. Alongside this, GnRH pulsatility within the PVN and ARC is modulated by these hormones and so consequently, FSH and LH production and secretion from the anterior pituitary is impacted, thus resulting in reduced oestrogen production (Allaway, Southmayd & De Souza, 2016).

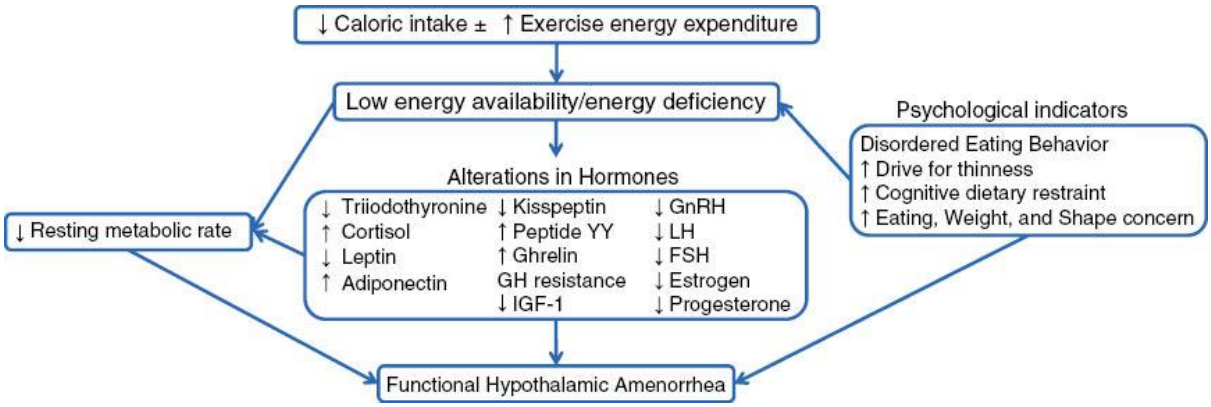

Figure 4: A diagram demonstrating the complexity of endocrine disruption caused by LEA and the alteration of each hormone (Allaway, Southmayd & De Souza, The physiology of functional hypothalamic amenorrhea associated with energy deficiency in exercising women and in women with anorexia nervosa 2016, volume 25(2), p 91-119).

GH, growth hormone; IGF-1, insulin-like growth factor 1; GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone.

Glucocorticoids and Catecholamines

GnRH may be suppressed by further hormonal abnormalities that are associated with FHA. Firstly, the hormones glucocorticoids and catecholamines are also known to inhibit gonadal function in response to the stress of exercise (Wheatley et al., 2012). Specifically, corticotrophin releasing hormone (CRH) as mentioned, growth hormone (GH) and insulin-like growth factor (IGF-1), thyroxine and melatonin could also play a role (Figure 2) (Mendelson and Warren, 2010).

Ghrelin

The hormone ghrelin is an anorexigenic peptide that stimulates appetite but reduces fat utilization and oxidation and also affects GnRH pulsatility (Barreiro and Tena-Sempre, 2004). Several studies have suggested that ghrelin can interact directly with hypothalamic neurons, and therefore leading to the suppression of the release of gonadotropin, thus impairing fertility. It has been suggested that this is an effect that is dependent of the oestradiol milieu (Yeo and Colledge, 2018). This is because the induced changes in ghrelin levels associated with an energy deficiency are an important factor involved in the suppression of the HPO axis (Hill, Elmquist & Elias, 2008., Budak et al., 2006). Evidently, ghrelin has been linked to reproductive function through both direct and indirect actions that alter GnRH pulsatility (Hill, Elmquist & Elias, 2008., Budak et al., 2006) and lead to the suppression of LH secretion and pulsatility (Scheid et al., 2013., Vulliemoz et al., 2004., Misra et al., 2005, Fernández-Fernández et al., 2004).

Although exercising or underweight amenorrheic patients are characterized by a significantly greater serum ghrelin elevation than those who remain with stable weight (De Souza et al., 2004), ghrelin is responsible for the prolongation of amenorrhea in subjects who have regained normal weight (Schneider and Warren, 2006). This is because ghrelin potentially serves as a biomarker of increased energy efficiency (*i.e.,* lower energy expenditure) in humans (St-Pierre et al., 2004), as administration has shown weight gain as a result (Wren et al., 2000), and suggests a slowing of the patients RMR. This offers an explanation as to why women who have regained normal weight or ceased exercise but still show distorted eating patterns, can have prolongation of amenorrhea as they still have low energy availability (Schneider and Warren, 2006).

Kisspeptin

Kisspeptin is a principal regulatory protein important for initiating secretion of GnRH, and its signalling has been implicated as the common intermediate signalling factor, acting downstream of leptin and other neuro-modulatory signalling systems to modulate activity of GnRH (McCarthy, 2013). Kisspeptin's regulatory role comes from binding to GnRH neurons to increase gonadotropin release, with a preferential stimulatory effect for the release of LH (Skorupskaite, George, Anderson, 2014., Chan et al., 2012., Dhillo et al., 2007., George,

Anderson, Millar, 2012., Jayasena et al., 2014., Jayasena et al., 2013., Jayasena et al., 2013., Jayasena et al., 2010., Jayasena et al., 2009), as administration of kisspeptin54 to women with FHA stimulated an increase in LH pulse frequency (Jayasena et al., 2009., Jayasena et al., 2010., Jayasena et al., 2014). When there is a notable reduction in GnRH secretion as a result of food deprivation, this is found to be coupled with a reduction in kisspeptin secretion (Castellano et al., 2005., Castellano et al., 2013., Luque et al., 2007., Smith et al., 2006).

Adiponectin

More recently adiponectin, a hormone of which levels rise with prolonged fasting and weight reduction, has been determined to reduce basal and GnRH-stimulated LH secretion (Rodriguez-Pacheco et al., 2007), and so high levels of adiponectin are present in energy deficient female athletes (Roupas and Georgopoulos, 2011).

2.3 The Female Triad and RED-S

2.3.1 The Female Athlete Triad

The Female Athlete Triad (Figure 4) is defined as a clinical entity that refers to the relationship between three interrelated components: energy availability (EA), menstrual function and bone health (Nativv et al., 2007). Although any one of these components can occur in isolation, LEA often begins a cycle in which all three occur in sequence - hence the entity being described as a "Triad" (Mountjoy, 2014). LEA causes a decrease in endogenous oestrogen (Figure 2 and 3), which can eventually result in an imbalance in bone remodelling leading to low bone mass or osteoporosis (Ilhe and Loucks, 2004).

The pathophysiology underpinning the Female Athlete Triad is described as a continuous spectrum over a period of time, where the athlete can move from either end. It ranges from the healthy athlete with optimal EA, regular menses, and healthy bones to the opposite end, characterised by amenorrhea, low EA and osteoporosis (Figure 4) (Nativv et al., 2007). Further research has determined that this phenomenon is not a triad but a syndrome resulting from relative energy deficiency and affects many aspects of physiological and psychological health. Therefore, it was suggested that each component of the Triad develops on a continuum and that there are Triad "stages" (Figure 1). If the early stages are not treated properly, people can progress toward the extremes of the Triad (De Souza, 2003). Therefore, the prevalence

of the Female Athlete Triad and each of its components can range extensively in sports populations due the spectrum of severity that the entity covers.

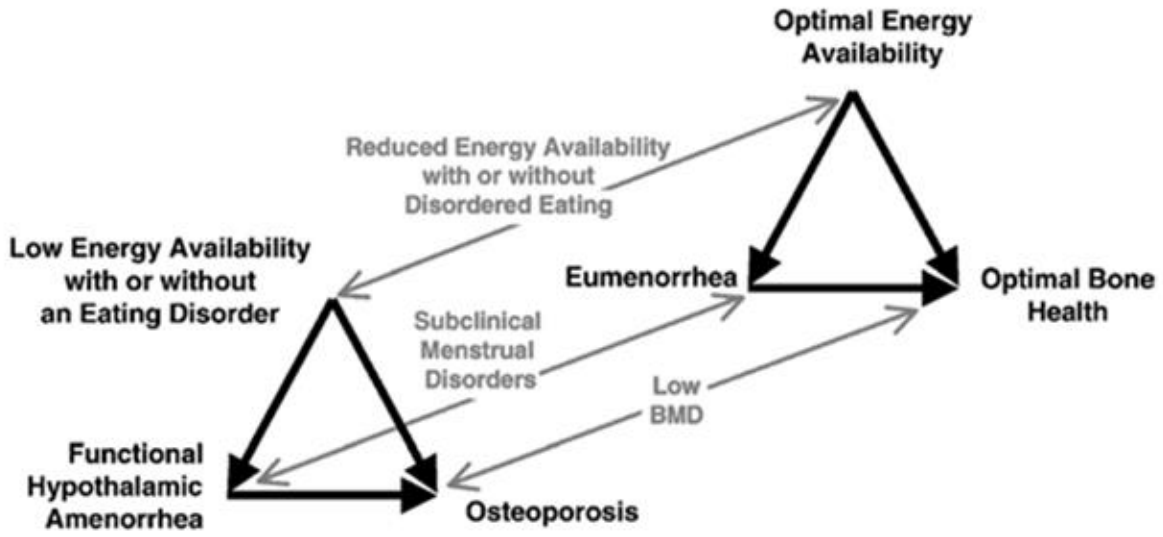

Figure 4: The Female athlete triad (Nattiv et al., The female athlete triad: ACSM position stand 2007, volume 39, p 1867-1882). The ideal female athlete is to the far right of the spectrum, which defines optimal health.

According to the American College of Sports Medicine, females who participate in sports that emphasise low body mass are at the greatest risk for developing one or more of the components of the Female Athlete Triad, including disordered eating (Otis et al, 1997). These include but are not limited to gymnastics, dance, swimming, and endurance-based events such as long-distance running. This increased risk is sometimes due to the pressure placed on women to achieve or maintain an unrealistically low body mass, particularly in sports that emphasise a small physique and leanness. More recently, studies (Clark et al., 2018) have shown that the Female Athlete Triad occurs frequently amongst athletes who engage in sporting events whereby the physiological and/or athletic requirement for an excellent performance is hinged on maintaining a low percentage of body fat. In many cases this pressure can lead to low energy availability (LEA) in an individual and so leads to the development of components of the Female Athlete Triad (Otis et al, 1997).

A systemic review of nine studies (n=991) reported the prevalence of the Triad for one, any two and all three components. The prevalence of one component of the Triad ranged from 16 to 60%, the prevalence of any two Triad components ranged from 2.7 to 27%, and the prevalence of all three Triad conditions presenting simultaneously ranged from 0 to 16% (Gibbs et al, 2013). Likewise, the review stated that participation in a lean or aesthetic-based sport increased the prevalence of each combination of Triad components. As a result of the emphasis placed on low body mass in many different sports, several small trials suggest that the prevalence of disordered eating amongst female athletes could be as high as 62% in some sports (West, 1998). Notably, endurance athletes for example, can also inadvertently reduce their calorie intake and therefore energy availability through large amounts of daily exercise, whilst continuing to consume the same diet as someone sedentary (Edwards et al, 1993). Therefore, many athletes can experience components of the Female Athlete Triad as a result of LEA often when they go through an intense period of training and may not adjust their calorie intake accordingly, rather than just through self-restrictive practices.

2.3.2 Relative Energy Deficiency in Sport

The definition of the Female Athlete Triad is very narrow, and it does not incorporate the variety of physiological conditions associated with LEA. As well as this it fails to acknowledge similar pathophysiology in men. As a result, the International Olympic Committee introduced the term RED-S, an abbreviation for Relative Energy Deficiency in Sport, in order to expand on the concept of the Female Athlete Triad and acknowledge a wider range of outcomes as well as its application to male athletes (Mountjoy et al., 2014). RED-S is defined as the impaired physiological functioning caused by relative energy deficiency and includes, but is not limited to, impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis and cardiovascular health (Figure 5) (Mountjoy et al, 2014). Figure 5 highlights the expanded concept of the Female Athlete Triad, and where it fits into RED-S, aiming to acknowledge a wider range of outcomes as well as acknowledging those seen in men. The cause of RED-S is energy deficiency, relative to the balance between dietary energy intake and energy expenditure required for health and activities of daily living, growth, and sporting activities. Therefore, the aetiological factor of this syndrome is low energy availability (LEA) (Mountjoy et al, 2014). LEA occurs due to a reduction in energy intake (EI) and/or an increased exercise load and causes adjustments to body systems to reduce energy

expenditure, leading to a disruption of hormonal, metabolic and functional characteristics (Loucks, 2004).

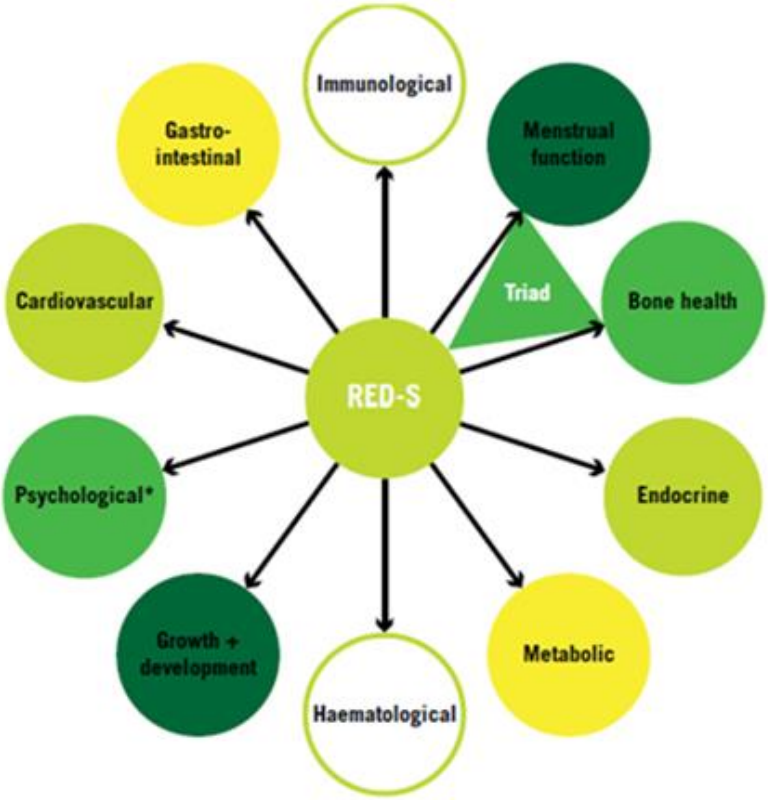

Figure 5: A summary of the health complications caused by RED-S (Mountjoy et al., The IOC consensus statement: beyond the female athlete triad—relative energy deficiency in sport (RED-S) 2014, volume 48(7), p 491-497).

*Psychological consequences can either precede RED-S or be the result of RED-S.(Mountjoy et al, 2014)

As RED-S encompasses more health complications than the Female Athlete Triad, it has a much higher prevalence amongst the athletic population, ranging anywhere from 6% -100% depending on the sport (Logue et al, 2018). Similarly, to the Triad, a high prevalence of LEA is observed in individuals participating in sports where a low body mass and leanness is desirable as it is believed to improve performance or appearance, of which are concomitant with the high energy demand of the training regime (Dipla et al, 2020). In addition to this, significantly more athletes participating in individual sports are at risk of LEA compared to those partaking in team sports (Slater et al., 2016).This is potentially due to the nature of

many individual sports being more reliant on body image and size, for example gymnastics in comparison to a team sport like football.

As a result of these complications, LEA and hence RED-S, can have a detrimental effect on athletic performance (Figure 6) as a result of the many health complications. For example, LEA-related menstrual dysfunction is associated with increased bone stress injury risk which can impair training and competition availability (Enns and Tiidus, 2010). Thus, low EA may be a contributor to poor sports performance due to associated detrimental endocrine effects (Elliott-Sale et al., 2018). Likewise, there can also be a decrease in neuromuscular performance, and this was observed in elite endurance athletes with menstrual dysfunction in contrast to eumenorrheic endurance athletes (Tornberg et al., 2017). The decreased neuromuscular performance was associated with lower fat free mass in the leg, glucose, oestrogen, T3, and elevated cortisol. While these findings are unable to provide sufficient evidence of a causal link between these biomarkers and performance, the interrelationship is biologically possible. This is because it is possible that consistently low blood glucose levels may lead to increased cortisol and reduced T3, in addition to lower muscle mass in the long term (Tornberg et al., 2017). Therefore, RED-S encompasses these elements of sports performance not previously identified in the Female Athlete Triad.

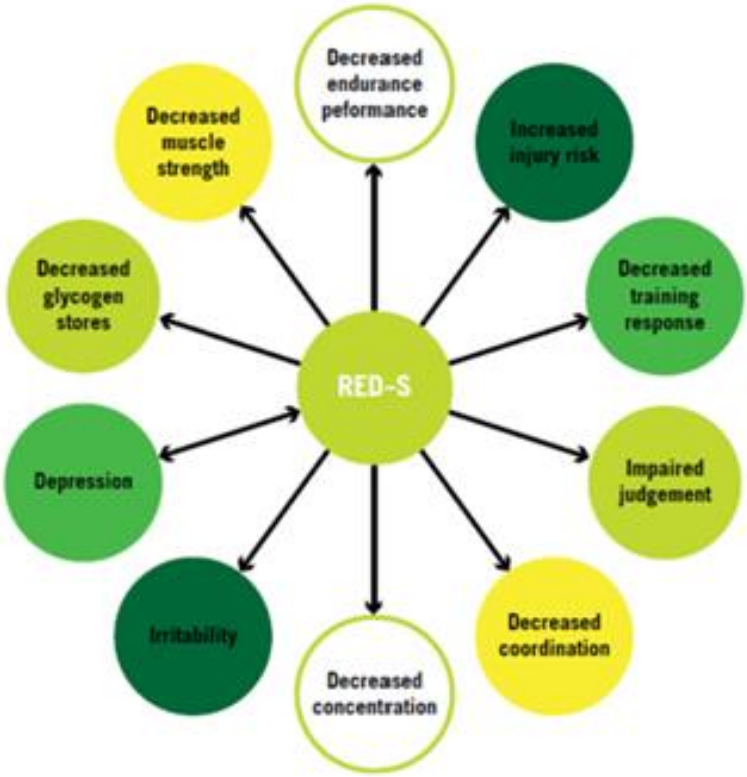

Figure 6: The sports performance consequences associated with RED-S (Mountjoy et al., The IOC consensus statement: beyond the female athlete triad—relative energy deficiency in sport (RED-S) 2014, volume 48(7), p 491-497).

2.4 Bone Health

Healthy athletes typically have bone mineral density (BMD) that is 5% to 30% greater than their sedentary counterparts (Nichols et al., 2000. Nichols et al., 2007). Maintenance of this elevated BMD could lead to a 50 to 80% fracture risk reduction (Johnston and Slemenda, 1994. Kanis et al., 2001. Nordstrom et al., 2005. Nichols et al., 2007). However, many female athletes are at risk of developing the FAT or RED-S as a result of LEA, which can lead to poor bone health. Poor nutritional intake and therefore LEA, which leads to impaired menstrual function and impacts bone health, attenuates the beneficial effects of exercise. Therefore, female amenorrheic athletes have lower bone mineral density (BMD) than eumenorrheic athletes and nonathletic controls (Russell et al., 2009), increasing their risk for fractures.

Bone tissue is dynamic and constantly being remodelled by osteoclasts, which resorb old bone, and osteoblasts, which form new bone (Feng and McDonald, 2011). This is done under the control of polypeptides, steroid hormones, thyroid hormones, cytokines, and growth factors (Ackerman and Misra, 2011). LEA has independent negative effects on reproductive function and bone health, alongside low levels of gonadal steroids also being detrimental to bone health. Amenorrheic and Oligomenorrheic female athletes function at a low oestrogen state, and oestrogen's principal role in bone is to directly act on osteoblasts, and acting indirectly on osteoclasts to prevent bone resorption. Therefore, a hypoestrogenic state, due to LEA, cause disruption of bone remodelling and accelerated bone resorption by osteoclasts (Riggs et al., 2003) and reduced BMD. Moreover, low body mass may also decrease BMD (Joy, Kussman and Nattiv, 2016). Currently, the age of peak bone mass acquisition is approximately 18–20 years, and if low oestrogen status or low body mass is present at puberty, it is not possible to acquire maximal bone mass, leading to low BMD/osteoporosis (Matkovic et al., 1994. Russell and Misra, 2010).

Athletes should have a 5% to 15% higher bone mineral density (BMD) than age-matched nonathlete (Movaseghi et al., 2012). Altered bone mineral density will increase bone fragility and increase the likelihood of osteoporosis and the risk of fractures (Raisz and Rodan, 2003). The incidence of stress fractures is 2 to 4 times greater in amenorrheic athletes than eumenorrheic controls (Joy, Kussman and Nattiv, 2016) , and bone density has been shown to negatively correlate with the number of missed menstrual cycles since menarche (Raj, Creech and Rogol, 2020), demonstrating the tight link between hypoestrogenism and bone health (Figure 4). Therefore, understanding the mechanism for bone loss in exercising women with LEA may be as a result of independent effects of hypoestrogenism on bone.

2.4.1 Bone Mass in Amenorrheic and Oligomenorrheic Athletes

BMD in Amenorrheic and Oligomenorrheic Athletes

Many early studies have investigated the influence of menstrual status on bone mass in women of reproductive age (Prior et al., 1990. Carbon, Pettersson et al., 1992. and Drinkwater et al.1999), showing that amenorrheic women of reproductive age had lower BMD than women with regular menstrual periods, and that the bone loss was more notable in the lumbar spine than in other parts of the skeleton. The lack of difference in femoral neck BMD

often noted in earlier studies was noted to be as result of the faster bone remodelling process in trabecular bones, such as the lumbar spine, than in cortical bones (Petterson et al., 1999). In order for the effects of amenorrhea to be expressed in cortical bone, it has been suggested that a duration of 5–6 years of amenorrhea was necessary (Myerson et al., 1992). This highlights the notion of menstrual disturbances and the extent of their complications as being on a continuum, with longer term health implications dependent on duration and extent of LEA.

Drinkwater et al., (1986) stated that 'the amenorrhea is the factor that determines bone loss, but the age at onset of amenorrhea, especially when the amenorrhea appears before women reach their peak bone mass, is of greater importance than the duration of amenorrhea'. Although this is still relevant, as maximal bone mass acquisition takes place between the ages of 18-20, this could now be updated to suggest that it is the age at which a female athlete first has LEA as opposed to amenorrhea, as the defining factor in BMD. This is because the metabolic imbalances that LEA causes is what initiates menstrual disturbances. However, it is also important to consider other factors, such as stress when determining the initial catalyst for an athlete's menstrual disturbance.

Although the most severe bone loss has been associated with amenorrhea, oligomenorrhea has also been associated with low BMD in female athletes, and therefore, less severe menstrual disturbances should not be dismissed clinically (Tomten et al., 1998. Cobb et al., 2003). Cobb et al., (2003) reported that oligomenorrheic athletes have a lumbar spine BMD that is only 69% of that observed in an aged-matched cohort of menstruating controls. Likewise, it is important to note that not only is BMD associated with an individual's current menstrual status, whether that be amenorrheic or oligomenorrheic, but also their history of menstrual disturbances, as shown by Drinkwater et al. (1990). Therefore, there can be a cumulative impact of decreased endogenous oestrogen over many years.

Fat Deposits

Regional fat deposits, such as subcutaneous, visceral and marrow fat, have been implicated in the regulation of bone mass at extremes of LEA (Ackerman et al., 2016). In adult females, visceral adipose tissue (VAT) has deleterious effects on bone, especially on femoral and

cortical bone areas, whereas subcutaneous adipose tissue (SAT) has a positive association with bone mass (Gilsanz et al., 2009). Marrow adipose tissue (MAT) has a common progenitor mesenchymal stem cell lineage with osteoblasts. Therefore, many osteoporotic states such as anorexia nervosa are associated with decreased BMD and increased MAT (Bredella, 2009), but this is still a developing area of knowledge.

Caloric restriction alone, which is putting the body into a state of LEA, but in the absence of changes in oestrogen, has been shown to be an important factor in bone loss (Shapses et al., 1998). This is further evident in studies where oral contraceptive use in patients with anorexia or in women with exercise-associated amenorrhea is not associated with complete recovery of BMD (Zipfel et al., 2001). Similarly, resumption of menses in formerly amenorrheic athletes does not result in complete recovery of BMD, which has also been shown to decrease further in amenorrheic athletes if left untreated (Drinkwater et al., 1986. Drinkwater and Keen, 1997). Therefore, in addition to considering the degree of hypoestrogenism as a contributor of bone loss in amenorrheic athletes, the impact of LEA alone can also be pursued as a potential cause of diminished BMD. This may be because, as previously mentioned, fat deposits have deleterious effects on bone, and are implicated at extremes of LEA, suggesting a role for changing fat deposits in low BMD.

Contrastingly, it has been shown that weight gain in women with anorexia nervosa was associated with significant central fat accumulation relative to the extremities (Grinspoon et al., 2001). This redistributed weight gain may offer an explanation as to why BMD can remain low in previously amenorrheic athletes that have gained weight, highlighting a further role for fat deposits in their influence over bone mass. Although further research is needed to investigate the link between fat deposits and BMD in amenorrheic athletes, there is evidence of a link.

The Oestrogen Pathway

Oestrogen deficiency, a known cause of low BMD, increases adipocyte differentiation, and rodent studies have demonstrated a dose-related decrease in MAT following oestrogen administration (Gao et al., 2014), suggesting a potential interplay between fat deposits and

hypoestrogenism that promotes bone resorption. The benefits of exercise include increases in both muscle mass and BMD; however, it has been concluded that the beneficial effects of weight-bearing exercise on BMD in eumenorrheic athletes are attenuated in oligomenorrheic and amenorrheic athletes as a result of hypoestrogenism (Nichols et al., 2007. Ackerman et al., 2011).

Findings from a study by Drinkwater et al. (1990) demonstrated that as menstrual irregularities became more severe, the negative association between body mass and bone health became stronger. This suggested an important interaction between menstrual pattern, body mass and vertebral density, highlighting a potential role for energy balance in menstrual disturbances. Further to this, the study also reinforces that LEA influences menstrual disturbances and causes hypoestrogenism, which further leads to a decline in bone health.

The Insulin-like Growth Factor (IGF)-Pathway

IGF-1, a nutrition-dependent factor that stimulates osteoblast function and bone formation, is a useful factor to measure, especially in cases of FHA, low bone mass (Gordon et al., 2001). Optimal bone accrual during adolescence and early adulthood is dependent on the rising levels of IGF-1, and the antiresorptive and also bone anabolic effects of rising sex steroids (Soyka et al., 2000), highlighting an intertwining link between the HPO axis and IGF-1 (Figure 2).

The effects of caloric restriction, and therefore LEA, may independently affect bone through energy deficit-induced decreases in bone trophic factors, such as IGF-1 (Zanker and Cooke, 2004). It has been identified that the lowest levels of the bone formation markers were observed in the amenorrheic athletes with the lowest total T3 and IGF-1 (Zanker and Swayne, 1998. Ihle and Loucks, 2004.) .

Implications for athletes

For athletes, low BMD increases the risk of stress fractures and osteoporosis in later life (Myburgh et al., 1990), meaning that they would not be able to compete or train to their full potential. The setbacks of injury would impede athletic performance (Figure 6), which could form part of a vicious cycle where athletes take greater measures, whether this is increasing

exercise or reducing calorific intake, to achieve their goals. Likewise, it is also important to bear in mind that athletes with menstrual disturbances who compete in non-weight bearing sports may experience greater detrimental effects on their bone health than those in weight bearing sports, as this can attenuate, to an extent, low BMD caused by LEA.

2.5 Cardiometabolic health

The cardiometabolic health of amenorrheic athletes is an aspect of FHA that has not been extensively documented nor understood. This is particularly due to the lack of longitudinal studies and therefore the long-term cardiovascular consequences that amenorrheic athletes face. It has been confirmed that the oestrogen E2 has a cardio-protective role, and therefore hypoestrogenism, which is the manifestation and accumulation of the final consequences of complex hormonal changes from FHA, would result in cardiovascular disease risk factors (Hoch et al., 2003). Therefore, the independent and combined effects of chronic hypoestrogenism and exercise, together with subclinical dietary behaviours typically observed in amenorrheic athletes, warrants closer examination (O'Donnell and De Souza, 2012).

2.5.1 Oestrogen and Cardiometabolic Health

Hypoestrogenism

As both amenorrheic athletes and postmenopausal women are hypoestrogenic, studies in postmenopausal women can be used as a point of comparison to determine the potential cardiometabolic implications of FHA. This is because there are currently no existing long-term studies of cardiometabolic health in amenorrheic athletes. However, the altered vascular health outcomes reported in amenorrheic athletes in the short term, alongside postmenopausal comparison studies, are suggestive of increased risk for premature cardiovascular disease (CVD) (O'Donnell and De Souza, 2012). Hypoestrogenism in postmenopausal women is associated with unfavourable effects upon serum lipids (Ridker et al., 2000) endothelial function (Lieberman et al., 1994) haemostatic parameters (Rosenson et al.,1998) blood flow (Moreau et al., 2003) homocysteine (Yildirir et al., 2002) and antioxidant status (Yen et al., 2001) as a result of hypoestrogenism.

Recent data in amenorrheic athletes demonstrate impaired endothelial function (Hoch et al., 2003), elevated low- and high-density lipoprotein levels (Drinkwater et al., 1993), reduced circulating nitrates and nitrites (Stacey et al., 1998), and increased susceptibility to lipid peroxidation (Ayres et al., 1998) as cardiovascular consequences of hypoestrogenism. Predictive serum markers of cardiovascular health, such as homocysteine and C-reactive protein, are yet to be assessed in amenorrheic athletes, but are elevated in postmenopausal women (O'Donnell and De Souza, 2012). Although the clinical significance is not known, these markers are also suggestive of an increased risk of premature CVD.

Once a protective cardiovascular role for oestrogen (E2) had been identified, impaired cardiovascular health was suggested as an additional risk for hypoestrogenism in female athletes (Hoch et al., 2003). Oestrogen is known to decrease low-density lipoprotein (LDL) oxidation and the accumulation of oxidized LDL in the intima, which are crucial steps in the atherosclerotic process. Atherosclerosis is a process in which blood, fats such as cholesterol and other substances build up on your artery walls. Eventually, deposits called plaques form which can eventually lead to blood clots, and therefore oestrogen helps prevent this from occurring (Rickelunde et al, 2015). Although endogenous E2 is considered to be cardioprotective, the Women's Health Initiative trial that used hormone replacement therapy (HRT) on postmenopausal women identified a significantly increased risk of adverse cardiovascular events (Wenger, 2003). Therefore, although comparing amenorrheic athletes to postmenopausal women can be a highly useful comparison, it is important to consider that the administration of exogenous hormones to postmenopausal women is likely to result in different outcomes than endogenous E2 in premenopausal women, due to their stage in life and bodily requirements (O'Donnell and De Souza, 2004). A cardioprotective effect of endogenous oestrogen in premenopausal women is widely supported, however what remain less clear are the implications of persistently low oestrogen levels in much younger amenorrheic athletes.

2.5.2 Cardiometabolic health in Amenorrheic athletes

2.5.2.1 Lipid Metabolism

Low Density Lipoproteins

Low Density Lipoproteins (LDLs) have a key role in the transportation of cholesterol to all tissues, but primarily to adipose cells and the liver (Feingold & Grunfeld, 2012). Approximately 60–80% of circulating LDL-C is taken up by the liver via receptor-dependent mechanisms (Dietschy, 1997). Apolipoprotein B, the major protein moiety of LDL, acts as the ligand to the LDL receptor in the liver (Liscum & Munn, 1999), enabling the mechanisms to take place.

Dietary cholesterol and fatty acids consumed influence the circulating levels of LDL-C in the body, mediated by altering either hepatic (liver) LDL receptor activity, LDL-C production rate, or both. When the intake of dietary cholesterol is increased, expansion of the pools of newly synthesised sterol occurs within liver cells. This results in the downregulation of the LDL receptors, causing a plasma increase in the concentration of LDL-C (Dietschy, 1997). If elevated serum levels of LDL-C are greater than 3.37 mmol/L, this is recognised as an independent risk factor for CVD (NIH Consensus statement, 2003).

Studies observing LDL-C levels (Kaiserauer et al., 1989. Friday et al., 1993. Thompson et al., 1997. Ayres et al., 1998. Baer. 1999) and LDL particle size (Kaiserauer, 1989) in amenorrheic athletes have been reported, but findings are questionable, due to variable methodologies and small sample sizes. The contradictory literature currently available on this highlights that LDL-C levels have been both reported to be significantly elevated (Friday et al., 1993. Kaiserauer, 1989), and reported as non-significant differences (Baer et al., 1999. Akahoshi et al., 2001) in amenorrheic compared with eumenorrheic athletes. Therefore, it is not clear whether the reported elevation in LDL-C levels seen in amenorrheic athletes are of clinical significance. However, in an extensive review of postmenopausal women, Schwertz and Penckofer (2001) identified that 25–50% of the potential cardioprotective effect of E2 is associated with its effect on blood lipids and lipoproteins, suggesting that these levels are disrupted in a hypoestrogenic state. This identified range of potential cardioprotective effects

may potentially suggest that the differing duration of amenorrhea in the patients tested may impact the clinical significance of the LDL level reported, highlighting that the extent of menstrual disturbances move along a continuum (Figure 2).

Further to this, studies that report elevated LDL-C levels in amenorrheic athletes (Friday et al., 1993. Kaiserauer, 1989) also either show statistical significance, present in the Kaiserauer study, or a strong trend, present in the Friday study, for both reduced calorie intake and dietary fat intake. The paradoxical increase in circulating LDL-C despite reduced dietary fat intake in these studies is consistent with findings in chronically hypoestrogenic anorexia nervosa patients (Feillet et al., 2000). This opposes the typical functioning previously described, where an increase in cholesterol and dietary fat leads to increased LDL plasma levels. Mechanisms for this phenomenon in the anorexia nervosa patient group are associated with the known down-regulatory effect of altered thyroid hormones, i.e., reduced total triiodothyronine (T3), and lowered E2 levels (Feillet et al., 2000) on the cellular number of hepatic LDL receptors. As a result, this contributes to increased plasma LDL-C levels (Homma et al., 2002). Therefore, it is possible that this is one of the body's coping mechanisms for adjusting to LEA .

Similarly, to the anorexia nervosa group, amenorrheic athletes have also displayed low total T3 status (Loucks et al., 1992. Thong et al. 2000), a lower calorie intake (Kaiserauer, 1989) and significantly less calories derived from dietary fat (Drinkwater et al., 1993) when compared to the eumenorrheic controls. These studies suggest that, although to a lesser extent than those observed in anorexia nervosa patients, the metabolic aberrations observed in amenorrheic athletes may explain their elevated LDL-C levels (O'Donnell and De Souza, 2004).

As previously discussed, the findings provide evidence for the suggestion that dietary restriction and therefore LEA are placed upon a moving continuum, as amenorrheic athletes that have the greatest nutritional aberrations demonstrate the least favourable LDL-C profiles. Likewise, along this continuum time may also interplay, as it is possible that the longer the episode of amenorrhea, the greater the risk of elevated LDL-C.

Furthermore, De Souza and O'Donnell (2004) suggest that the downregulation of LDL receptors due to hypoestrogenism and altered thyroid status may also play a role in cholesterol metabolism in amenorrheic athletes, but this has not been thoroughly researched.

High Density Lipoproteins (HDL)

HDL particles correlate inversely with the risk of CVD (Roeters van Lennep, 2002) and are anti-atherogenic (Spieker et al., 2002), highlight its cardio-protection properties. HDL has a role in reverse cholesterol transport whereby it scavenges surplus cholesterol from peripheral tissues for delivery to and disposal by the liver for excretion via bile. This involves apolipoprotein A-I, one of the two major proteins associated with HDL (Guetta et al., 1996). Reverse cholesterol transport is important for cellular cholesterol homeostasis, and it is a result of apolipoprotein A-I promotion of cholesterol efflux from the cells via receptor and non-receptor mediated mechanisms (Segrest et al., 2000). The antioxidant properties of HDL are shown through studies where HDL attenuates LDL oxidation and inhibits the atherogenic effect of oxidised LDL-C (Bonnefront-Rousselot et al., 1999), which contribute to healthy endothelial function.

Reductions in HDL-C levels is notable after menopause, potentially due to diminishing levels of E2. E2 has stimulatory effects on apolipoprotein A-I as well as beneficial roles in HDL-C metabolism and modifying HDL-C levels, composition and distribution, and augmenting cholesterol efflux (Ulloa et al., 2002). However, similarly to LDL levels, study findings are variable in amenorrheic athletes, including significantly elevated (Drinkwater et al., 1993), similar (Ayres et al., 1998) and non-significant trends toward lower (Baer, 1999) HDL-C concentrations in amenorrheic compared with eumenorrheic athletes. However, endurance-trained female athletes possess much higher HDL-C levels compared with sedentary women (Podl et al., 1994) and eumenorrheic and amenorrheic athletes also demonstrate significantly increased HDL-C levels compared with eumenorrheic sedentary controls (Thompson et al., 1997). This suggests that exercise has cardioprotective benefits that may attenuate the risks of amenorrhea in some incidences, highlighting a reason for inconsistency in findings. This is supported through a study that found elevated HDL-C levels in female runners, irrespective of menstrual status, with the highest levels observed in those running the greatest distances

(Williams, 1996). This suggests that there are oestrogen-independent mechanisms effecting the HDL-C increase.

2.5.2.2 Glucose

The aetiology of FHA involves persistent LEA and therefore low glucose availability, which poses a variety of cardiovascular and muscular risks (Loucks and Thuma, 2003). Firstly, the inability to maintain sufficient glucose supply to the contracting muscle has been shown to decrease the intracellular calcium (Ca2+)concentration via a depressed release rate of Ca2+ from the sarcoplasmic reticulum(Gejl et al., 2014). In terms of athletic consequences, this is potentially contributing to the fatigue and delayed recovery in athletes with RED-S. Therefore, alterations in glucose homeostasis, alongside those seen in endogenous sex steroids, stress hormone levels and circulating thyroid hormones, may contribute to lowered neuromuscular performance in female athletes (Tornberg et al., 2017).

Cortisol is a steroid hormone released in response to starvation and intense exercise, resulting in a decrease in plasma glucose. This is because a major function of cortisol is to maintain plasma glucose concentrations by breaking down skeletal muscle into amino acids for gluconeogenesis by the liver. Therefore, an amenorrheic athlete with either or both LEA and an intense training routine, would have increased levels of cortisol in response to this stress, and would lead to a further reduction in blood glucose levels, inducing muscle atrophy and reduced athletic performance. A study by Tornberg et al. (2017) found that the amenorrheic athletes had lower blood glucose, T3, FFM, and higher cortisol as well as cortisol-to-insulin levels compared with the eumenorrheic control group and that this may all, directly or indirectly, contribute to the observed reduced neuromuscular performance. In addition to this, 86% percent of the amenorrheic athletes were hypoglycaemic, not only in the fasted and rested state but also after low-intensity exercise. Therefore, this reinstates that amenorrheic athletes have a general inability to maintain glucose homeostasis. As a result, blood glucose levels have been shown to influence athletic performance (Bangsbo et al., 1992). Energy supply is partly maintained by increased proteolysis, mainly from skeletal muscles, to produce free amino acids for the increased gluconeogenesis in the liver (Darmaun et al., 1988). Gluconeogenesis is the metabolic process by which organisms produce sugars (namely glucose) for catabolic reactions from non-carbohydrate precursors.

The reason for elevated cortisol levels in amenorrheic athletes are in response to a fall in insulin because insulin is important in the regulation of glucose metabolism and in the prevention of muscular proteolysis (Darmaun et al., 1988). Therefore, if proteolysis is not prevented due to a fall in insulin, gluconeogenesis would increase and therefore the levels of circulating blood glucose would decrease. This is supported by studies that show that glucose infusions suppress cortisol (Maclaren et al., 1999), linking low blood glucose levels to higher proteolysis and muscle atrophy, because a higher cortisol-to-insulin ratio has been shown to accelerate proteolysis (Myerson et al., 1991).

2.6 Summary

To conclude, the extent of energy availability an athlete possesses ranges from LEA to optimal EA on a continuum, alongside the period of time to which an athlete has experienced their particular degree of energy availability. This corresponds to the extent of menstrual disturbance the athlete experiences and the length of time that it occurs for. Therefore, the degree of severity of the menstrual disturbance corresponds to the health complications that the athlete experiences, such as low BMD, an unfavourable lipid profile and low blood glucose levels. These complications determine an athlete's ability to perform and will impact the quality and time in which athletes are able to dedicate to training and competing. This highlights how RED-S is an increasingly important area of research as more information about the clinical entity emerges. RED-S needs to better understood in order to be able to prevent, diagnose and provide help to those experiencing menstrual disturbances and LEA, whether through restricted eating or just because they use more energy exercising than they consume through dietary intake.

Lastly, although this remains an area not yet well understood, female athletes with prolonged FHA appear to be at a higher risk of cardiovascular complications in the future. Studies in pre-menopausal adult women have shown that having hypothalamic hypoestrogenism is associated with a higher risk of coronary artery disease (Merz et al., 2003). In addition, female athletes are also at risk of having abnormal lipid profiles, including elevated total cholesterol and low-density lipoprotein levels (Rickenlund et al., 2005) as a result of the down regulatory effects of reduced T3 and E2 levels. Heightened LDL plasma levels are an independent risk

factor for CVD. Dependent upon the type of exercise the athlete participates and the extent of their menstrual disturbance, HDL levels could either fall or show no significant difference, therefore further research is needed to determine the cardiometabolic risk to amenorrheic athletes with altered HDL profiles. As it is apparent that athletes in a state of LEA are at a greater risk of cardiovascular complications, it remains an important area of research in order to prevent the long-term complications associated with menstrual disturbances.

Chapter 3 - Methodology

3.1 Research Approach and Study Design

This research study adopted a positivist research ontological approach. This reflects the scientific enquiry nature of the study design and the quantitative results achieved, which were used to determine differences and correlations between the four study groups.

In this cross-sectional study comparing cardiometabolic risk factors and bone health, female athletes underwent a dual-energy x-ray absorptiometry (DXA) to assess bone density and body composition. Female amenorrhoeic athletes (AA), oligomenorrheic athletes (OA) and eumenorrheic athletes (EA) were included in this study.

3.2 Ethical approval

The study was approved by the NHS Ethics Committee (REC reference: 21/NE/0074) and the Department of Sport and Exercise Sciences Ethics Sub-Committee, with specific consideration to ionising radiation protection and ensuring compliance to Covid-secure protocols. All participants provided signed informed consent prior to participating in the study and the research was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Ethical approval granted by the NHS ethics Committee was for the use of DXA scans within this study. This is because DXA involves the utilisation of ionising radiation through low energy X-rays. The latest DXA scanners with fan beams, as used in this study, provides improved images for diagnostic radiographic quality. The effective dose for the current study 17 µSv (microsievert) which compares to 2.5 days of natural background radiation. Justification of effective dose is always required for DXA scans used in research and clinical practice, with a net benefit to the individual involved and the wider study. All participants received copies of the DXA results and had the opportunity to ask questions about the scan and their results.

COVID-19 Restrictions

Ethical approval was granted by the Department of Sport and Exercise Sciences Ethics Sub-Committee for all tests including finger-prick blood samples and the additional body measurements taken. This ethical review ensured that the rights, safety, dignity, and well-

being of research participants are safeguarded and, where applicable, legal requirements in sample legislation are met. Research only went ahead as the potential benefits outweighed any potential risks to the donors of the samples, as the finger-prick blood test is a relatively non-invasive procedure. The samples of human biological material were treated as donations and research involving these samples were conducted with respect and transparency. The ethical review was vital in ensuring that an environment of trust and respect with participants was created, recognising the altruism of providing samples for use (MRC ethics series, 2014). Unfortunately, however, due to COVID-19 restrictions this section of the study was unable to go ahead.

3.3 Study Sample

3.3.1 Sampling Methodology

Due to the travel restrictions as a result of Covid-19 and the specific requirements for participants in this research, non-probability (or non-random) sampling was used for this study. This is due to only certain groups of female athletes being able to take part because of the exclusion and inclusion criteria and their capacity to travel to Durham University. Therefore, recruitment of participants was based off of convenience and meant that if the participant met the exclusion and inclusion criteria, and was willing to travel, they could partake in the research. Therefore, this study doesn't allow for the generalization of the findings to other groups of females in wider settings.

More specifically, a voluntary response sampling method was undertaken. Details of the research project were broadcasted across universities, sports teams and the wider public with my contact details attached, and participants willing to be involved volunteered themselves. This was the most appropriate method of sampling as the research involved slightly more intrusive elements, with finger prick blood samples and DXA scans, and the research subject can be as a sensitive matter. Therefore, participant volunteering was deemed more suitable than randomly selecting individuals from each group and contacting them directly myself, due to the nature of the research conducted.

3.3.2 Exclusion and Inclusion Criteria

A total of 12 pre-menopausal female endurance athletes (aged19-42) were recruited from university and amateur cycling, athletics, and swimming clubs in the UK. Participants were grouped according to their menstrual cycle status. Four athletes were amenorrheic, 3 oligomenorrheic and 5 eumenorrheic. Participants were required to confirm that they were not pregnant before participating in the study as it would prevent them from receiving a DXA scan. Participants falling under the category of 'amenorrheic' would have had established menstrual cycles at some point, but at the time of the study they have had an absence of menstrual bleeding for 6 months or for a length of time equivalent to a total of at least 3 of her previous cycle lengths (West, 1998. Redman and Loucks, 2005). Those that fall under the category of oligomenorrheic have menstrual cycle intervals greater than 45 days (Mountjoy et al., 2014).

Participants were non-smokers, did not have a BMI that classified as overweight and have no history of medical conditions that impact bone health, such as: hypothyroidism, hyperthyroidism, diabetes mellitus, hypercortisolism, and renal or gastrointestinal disease. Participants were excluded if they used medications that may affect bone metabolism such as: oral contraceptives, Depo Provera, anabolic steroids, glucocorticoids, or anticonvulsants, in the preceding three months. Consumption of calcium and vitamin supplements were accepted into the inclusion criteria. Finally, participants also had no cardiovascular disease, or immediate family hereditary history.

It was also necessary to exclude women had a history of medical conditions impacting bone health, as well as those who had taken medications that affect bone metabolism in the three preceding months, as this study was looking at the effects of LEA on BMD. BMD is influenced by the success of bone metabolism as a continual cycle of bone growth and resorption. When energy intake is insufficient to meet the demands of exercise, luteinizing hormone (LH) pulsatility is weakened and results in a decline in oestrogen production, a hormone known to inhibit bone resorption (Loucks et al., 1998). Therefore, if there were any additional, external factors influencing bone metabolism, and thereby BMD, rather than LEA alone, the results may be distorted.

Participants also had to have no form of cardiovascular disease or a hereditary history in immediate family, as this study was investigating the impact of low energy availability on the cardiometabolic profile. In turn, this was used to determine whether athletes with menstrual disturbances were at an increased risk of cardiovascular disease (CVD) when looking at their lipid profile. Therefore, if there were external factors that increased the risk of CVD or altered the lipid profile, the results would have been impacted.

Study characteristics criteria	Participant			
	Amenorrheic Athlete	Oligomenorrheic Athlete	Eumenorrheic Athlete	Eumenorrheic Non-Athlete
Female, premenopausal non-smoker adults who aren't pregnant and don't have an overweight BMI				
No medical conditions that impact bone health				
No cardiovascular disease (or immediate family history?)				
No medication that may affect bone metabolism				
Complete a minimum of 3 hours of continuous aerobic exercise/week				
Primary sport is swimming, running, or cycling				
Menstrual disturbances				

Table 3: The inclusion and Exclusion Criteria for each group partaking in the study. Green represents the traits of the participants in each group, and red represents the traits that each group didn't have.

COVID-19 Mitigations

A sample size of 60-100 people was the initial aim for this study, however due to the impacts of covid-19 and a reduced timeframe to complete this project, the sample size was significantly reduced so that the study could still go ahead. Additionally, a comparison group of non-athletes that were eumenorrheic were also part of the initial study plan, being recruited from the University and the wider community throughout the UK. They would have been selected if they did not meet the requirements for the athlete status criteria determined, but they did meet the other inclusion/exclusion criteria

Participant Athletic Status

Athlete status and therefore the participants in this study, were required to be endurance athletes who complete a minimum of 3 hours of continuous aerobic exercise over the course of each week with their primary sport being either cycling, swimming or middle to long distance running. The 3 hours can be broken up into different amounts of time throughout the week, but the activity within that time must be continuous, i.e., 30 minutes of running.

3.4 Original Protocol

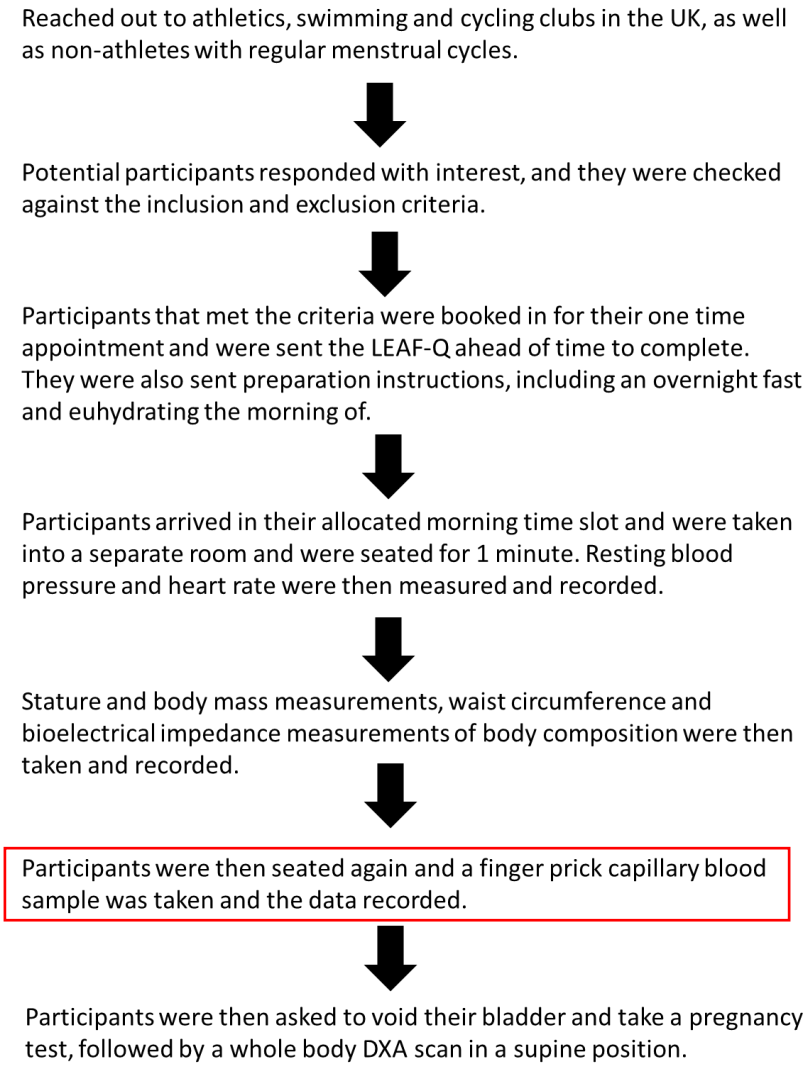

Figure 7: A flow diagram outlining the protocol conducted. The step outlined in red was the element not able to go ahead due to COVID-19.

Prior to arrival, energy availability was assessed using the validated Low Energy Availability in Females Questionnaire (LEAF-Q) (Melin et al, 2014), which was sent to participants via email as a link to a google forms questionnaire. The results were then collected and stored against each participant's unique ID number, alongside their results from the blood samples and the DXA scan.

Participants arrived at the laboratory at 08:00 in a euhydrated, rested state (no exercise that day), following an overnight fast of at least 8 hours. They were also instructed to abstain from caffeine and alcohol for 24 h before the appointment. To ensure participants arrived euhydrated they consumed 500 mL of water 1 hour prior to arrival time (Gibson et al., 2019).

Upon arrival, participants were taken one at a time into a separate room and were asked to remain seated for one minute, before measuring their resting blood pressure and heart rate. Following this, the stature and body mass of each participant was taken using a stadiometer (XX, United Kingdom) and calibrated electronic scales respectively (XX, United Kingdom). Stature was recorded in centimetres (cm) to the nearest millimetre and body mass, in kilograms (kg) to the nearest 0.1 kg. BMI (body mass/height2 [kg/m2]) was calculated for the purposes of comparison to obesity tables and the DXA results. A waist circumference measurement was taken and recorded to the nearest millimetre. Following these measurements, each participant then stepped onto a Tanita monitor to determine their bioelectrical impedance measurement of body composition, providing a bodily percentage of water and fat.

Participants were then asked to void their bladder and take a pregnancy test prior to the DXA scan. Participants wore light-weight clothing and removed their shoes and any jewellery prior to height and body mass measurements. Upon a negative pregnancy test result, a whole-body DXA scan was performed in a supine position.

3.5 COVID-19 restrictions

The body measurements would have originally been followed by a capillary blood sample collected from a finger prick, with all measurements being conducted immediately. Glucose would have been measured using the HB 201+ and HDL, triglycerides and total cholesterol levels were measured using the CardioChek PA. The format in which both pieces of equipment used for this analysis would have followed manufacturer's instructions. LDL levels were calculated using the results from the data collected by CardioChek PA.

Blood Sample Analysis (planned, but not performed)

Analysis of the blood sample would have determined each participant's fasting glucose, total cholesterol, high-density lipoprotein (HDL) and triglycerides measurements using blood chemistry analysers. It was planned to use the HB 201+ for fasting glucose, with measurements performed twice for accuracy. The HB 201+ is used in accordance with manufacturer's instructions where a new, separate microcuvette is used for every sample taken, with the correct disposal of each one afterwards. The test results appear after approximately 60 seconds.

The Cardiochek PA is to be in accordance with manufacturer's instructions. This involves the use of a new, separate test strip for each sample taken and the correct disposed of each strip afterwards. Test results appear within 90 seconds. Additionally, each day the Cardiochek PA should be checked for correct functioning using the grey check strip test and control solutions are used between batches of test strips.

These measurements are then used to calculate the individual's low-density lipoproteins using the Friedewald equation (Friedewald, Levy & Fredrickson., 1972):

$$\text{LDL-c (mg/dL)} = \text{TC (mg/dL)} - \text{HDL-c (mg/dL)} - \text{TG (mg/dL)}/5$$

LDL= Low-density lipoproteins
TC= Total Cholesterol
HDL= High-density lipoproteins
TG= Triglycerides

3.6 Revised Protocol (in light of COVID-19 restrictions)

Bone density, body composition and visceral fat measurements

Narrow fan beam dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Madison, WI) was used to evaluate left total hip BMD, left femoral neck BMD, anterior-posterior lumbar spine BMD (L1-L4) and total and regional body composition (lean mass, fat mass and visceral

fat mass). BMD Z-scores were produced for each of the 3 regions, with precision estimates (coefficient of variation) of 0.4% for lumbar spine BMD and 0.9% for femoral neck BMD. Precision errors for lean and fat mass measurements are 0.5% and 0.9%, respectively (mean age, 25 years) (Hind, Oldroyd & Truscott., 2011). BMD Z-scores are the number of standard deviations above or below the mean for the patient's age, sex, and ethnicity. Normal BMD is a Z-score greater than -1 in all measured sites and a Z-score of less than -2 in at least one of the measured sites indicates osteoporosis (Lewieki et al., 2004).

Daily calibration checks and DXA quality control observations were carried out prior to each scanning session using the GE Lunar calibration phantom and aluminium spine phantom. The DXA machine operator followed the manufacturer's guidelines on patient positioning and scan acquisition for the duration of the data collection (GE Healthcare, 2020), with identical scanning parameters used for each individual's scan. There was (no significant) drift in calibration for the study period during which the 12 athletes were measured.

The results for each individual were then compared to tables for age-matched and sex-specific reference intervals for iDXA derived VAT mass and the BMD Z-scores in adults of both the general population and athletes by using UK reference population data (GE Lunar Encore V.15.0, GE Healthcare, Madison, Wisconsin).

3.7 Statistical Analysis

3.7.1 Independent and Dependent Variables

The independent variables in this study were menstrual status and athletic status of the population selected. The study split the participants into three groups, amenorrheic athletes, oligomenorrheic athletes and eumenorrheic athletes with statistical tests also compared two groups, with menstrual disturbances as a collective group and eumenorrheic athletes.

The dependent variables in this study, within multiple different analyses were BMD Z-scores for total BMD, total hip, femoral neck, and posterior lumbar spine; BMI, body water percentage, body fat percentage, android:gynoid ratio, waist:height ratio, waist:hip ratio, waist circumference; resting blood pressure and heart rate; and body composition.

Dependent variables would have also included glucose, HDL, total cholesterol and LDL levels if the blood tests were able to take place.

3.7.2 Statistical tests

Statistical analyses were performed using the software SPSS version 27 (Armonk, NY: IBM Corp). Body composition variables (body water percentage, percentage body fat, android:gynoid ratio, BMI, waist circumference, waist:height ratio, waist:hip ratio, VAT) and bone density variables (total body BMD, total hip BMD, femoral neck BMD and anterior-posterior lumbar spine BMD), were first tested for data distribution formally by using the Shapiro Wilks test for normality. All variables were normally distributed, therefore parametric tests were used. Descriptive statistics (mean and ± standard deviation) were reported for each of the three groups.

Independent T-Tests were performed to determine whether there was a significant difference firstly between the menstrual disturbances (including both amenorrheic and oligomenorrheic athletes) and the eumenorrheic athletes, and then more specifically between amenorrheic and eumenorrheic athletes. An independent T-test compare BMD Z scores for the menstrual disturbances and eumenorrheic group, and the amenorrheic and eumenorrheic group. Therefore, the amenorrheic and oligomenorrheic athlete groups were jointly and individually compared to those that were eumenorrheic to see if there was a statistically significant difference for each subject group. Independent T-tests were also carried out for each of the body composition variables in the same way.

Pearson's correlation test was used to determine whether any of the variables correlated, to help deduce whether a change in one variable may take place alongside a change in another, or whether a change in one variable may be causing the change in another. Correlations were tested between Waist:height ratio and BMD, BMD total body and body water percentage, BMI and total body BMD, PBF and BMD total body, BMD total body and BMD lumbar spine, BMD total body and VAT, and BMD total body and android:gynoid ratio, with all 12 participants used to determine a correlation. Statistical significance was identified as $p < 0.05$.

Chapter 4 – Results

4.1 Participant Descriptive

Table 4: Range for Participant Descriptive

Variable	Range	Mean	Standard Deviation
Age	19-42	25	7.68
Height (cm)	155.2-177.1	165.64	6.04
Weight (kg)	43.2-71.5	54.37	8.31
BMI (kg/m2)	16.3-25.2	19.73	2.67

4.2 Tests for Normality

The data for the body composition variables (Table 9 & 11); total body water, PBF, android:gynoid ratio, waist circumference, waist:height ratio, waist:hip ratio, lean mass, visceral fat mass and BMI were all normally distributed (p value ranges 0.074-0.905 & 0.068-0.944). The data for bone mineral density variables (Table 10 & 12); total hip, femoral neck, lumbar spine and total body Z-score were all normally distributed (p value range 0.2-0.957 & 0.085- 0.973).

4.3 Body Composition

TBW in the menstrual disturbances group was higher than the control group (Table 5), and higher in the amenorrheic group compared to the eumenorrheic group (Table 6), although this did not reach statistical significance.

Waist:height ratio was significantly lower (Table 6) in amenorrheic v eumenorrheic athletes (mean= 0.38 (SD-0.013) – 0.44 (SD-0.03), p=0.19). There were no significant differences between the combined menstrual disturbances group and eumenorrheic group (p>0.05). For waist:hip ratio, android:gynoid ratio and percentage body fat there was no significant difference between any groups (p=>0.05).

Table 5: Mean and Standard Deviation for body composition of Menstrual Disturbances and Control Group

Measurement	Participant Type						
	Menstrual Disturbances			Control Group			Normal Range
	N	Mean	SD	N	Mean	SD	
TBW (%)	7	57.79	3.113	5	55.384	5.18	45-60%
BMI (kg/m2)	7	18.96	2.28	5	20.82	3.04	18.5–24.9.
Waist:Hip Ratio	7	0.71	0.028	5	0.75	0.06	≤0.85
Body Fat (%)	7	22.31	5.46	5	24.72	7.45	14-31%
Android:Gynoid Ratio	7	0.57	0.21	5	0.72	0.28	≤0.8
Waist:Height Ratio	7	0.40	0.028	5	0.435	0.03	<0.5
Visceral Fat Mass	7	0.91	0.39	5	1.36	0.88	
Lean Mass (%)	7	74.197	5.113	5	71.884	6.934	70-90%

*waist:height ratio was significantly different in the amenorrheic v eumenorrheic athletes

Table 6: Mean and Standard Deviation for body composition variables of Amenorrheic, Oligomenorrheic and Eumenorrheic Athletes

Measurement	Participant Type									
	Amenorrheic			Oligomenorrheic			Eumenorrheic			Normal Range
	N	Mean	SD	N	Mean	SD	N	Mean	SD	
TBW (%)	4	59.24	2.99	3	55.85	2.42	5	55.38	5.184	45-60%
BMI (kg/m2)	4	17.65	1.03	3	20.70	2.45	5	20.82	3.04	18.5–24.9.
Waist:Hip Ratio	4	0.7*	0.02	3	0.73	0.03	5	0.75*	0.06	≤0.85
Body Fat (%)	4	19.15	4.57	3	26.53	3.38	5	24.72	7.45	14-31%
Android:Gynoid Ratio	4	0.47	0.11	3	0.7	0.27	5	0.72	0.28	≤0.8
Waist:Height Ratio	4	0.38	0.013	3	0.43	0.03	5	0.44	0.03	<0.5
Visceral Fat Mass	4	0.68	0.26	3	1.23	0.31	5	1.36	0.88	
Lean Mass (%)	4	77.13	4.253	3	70.293	3.361	5	71.884	6.934	70-90%

4.4 Dual Energy X-Ray Absorptiometry

4.4.1 Bone Mineral Density

Total body BMD Z-score was lower (Table 7) in amenorrheic v eumenorrheic athletes (1.3 v 2.5, p=0.004). There was no significant difference in total body BMD Z-score (Table 8) between the combined menstrual disturbances group and control group (p>0.05).

Lumbar spine (L1-L4) BMD Z-score was significantly lower (Table 7) in amenorrheic v eumenorrheic athletes (-1-1 v 0.1, p= 0.041).

Table 7: Mean and Standard Deviation for BMD in Amenorrheic, Oligomenorrheic and Eumenorrheic Athletes

Measurement		Participant Type									
		Amenorrheic			Oligomenorrheic			Eumenorrheic			Normal Range
		N	Mean	SD	N	Mean	SD	N	Mean	SD	
BMD Z score	Total Hip	4	0.5	0.87	3	1.3	2.25	5	1.0	0.57	>-1.0 (Mountjoy et al., 2015)
	Femoral Neck	4	0.2	-1.05	3	1.5	1.65	5	1.0	0.63	
	Anterior-Posterior Lumbar Spine	4	-1.1**	0.7	3	0.5	1.32	5	0.1**	0.75	
	Total BMD	4	1.3*	0.42	3	2.5	1.76	5	2.5*	0.45	

*Total body BMD Z-score was significantly different in the amenorrheic v eumenorrheic athletes

**Lumbar spine BMD Z-score was significantly different in the amenorrheic v eumenorrheic athletes

Table 8: Mean and Standard Deviation for BMD variables of Menstrual Disturbances and Control Group

Measurement		Participant Type						
		Menstrual Disturbances			Control Group			Normal Range
		N	Mean	SD	N	Mean	SD	
BMD Z score	Total Hip	7	0.8	-1.5	5	1.0	0.57	>-1.0
	Femoral Neck	7	0.7	1.4	5	1.1	0.75	
	Anterior-Posterior Lumbar Spine	7	0	1.27	5	0.1	0.752	
	Total BMD	7	1.8	1.24	5	2.5	0.451	

4.5 Associations between variables

Waist:height ratio

Waist:height ratio was positively correlated with total body BMD Z-score, r=0.741, p = 0.006. This demonstrates that as the waist:height ratio increases, this is strongly correlated with an increase in total body BMD Z-score.

Likewise, waist:height ratio was strongly, negatively correlated with TBW, r=0-.874, p = <0.001. This highlights that as waist:height ratio decreases, body water percentage increases, and further demonstrating that amenorrheic athletes have a lower waist:height ratio and a higher TBW percentage.

Total body BMD Z-score

Total body BMD Z-score was moderately and inversely correlated with TBW, r=0-.562, p = 0.047, suggesting that when body water percentage decreases, total BMD starts to increase.

Total body BMD Z-score was also strongly, positively correlated between with BMI , r=0.748, p = 0.005. This suggests that a lower BMI is often indicative of a lower BMD total body Z-score and vis versa.

Total body BMD Z-score was moderately, positively correlated with PBF, r=.682, p = 0.015.

Total body BMD Z score was strongly, but not perfectly positively correlated with the lumbar spine (L1-4) BMD Z-score, r=0.786, p = 0.0024.

BMD total body (Z score) was moderately positively correlated with android:gynoid ratio, r=.640, p = 0.25.

No correlations

However, there was no significant correlation between waist:height ratio and the lumbar spine (L1-L4), nor BMD total body (Z-score) and visceral fat percentage, or visceral fat percentage and waist:height ratio.

Chapter 5 – Discussion

This study aimed to examine the differences in both the skeletal profile and cardiometabolic health of female athletes with menstrual disturbances v eumenorrheic athletes. In doing so, the main findings were that there was a significant difference in the total body and lumbar spine BMD Z-score of the amenorrheic v eumenorrheic athletes, with amenorrheic athletes having lower Z-scores. The study also found that amenorrheic athletes had a total body water (%) result at the top end of the normality range, and that this is a variable that could be further investigated in a larger sample size. Due to the impacts of COVID-19, blood tests were not able to be carried out and so the cardiometabolic health of the athletes was not thoroughly investigated. However, determining the results of these blood tests would be an important area of research for future studies.

5.1 Bone Mineral Density

Total body Bone Mineral Density (Z score)

The Z-score is the BMD compared to an age matched score. A Z-score below -2.0 indicates that your bone density is lower than it should be for someone of your age. This was raised to -1.0 in athletes (Mountjoy et al., 2015). Neither the menstrual disturbances group nor the amenorrheic group had a Z-score low enough to fall outside of the normal range. However, when the adjustment for athlete Z-scores is taken into account, the amenorrheic group mean falls slightly outside the normal range for athletes, at -1.1.

Despite total body BMD Z-scores being in the normal range for all three study groups, there was a statistically significant difference between the amenorrheic and eumenorrheic athletes. This difference demonstrates a marked decrease in BMD when amenorrhea is an influencing factor. There is a possibility that the amenorrheic athletes' BMD Z-score is lower than the eumenorrheic group, but not below the normal range, as the impact of running may mask the effect of amenorrhea on BMD. This is a possibility as exercise can be osteogenic, with athletes typically having higher BMD, favourable adaptations to bone microarchitecture, particularly at weight-bearing sites, and greater bone strength than their sedentary counterparts (Scofield & Hecht, 2012).

A study (Piaseki et al., 2018) comparing amenorrheic and eumenorrheic female distance runners and non-athletic controls observed that amenorrheic runners had a lower bone mineral density in the trunk, lumbar spine, ribs and pelvis than eumenorrheic athletes and controls. In contrast, the tibia (shinbone) cortical bone strength indicators were greater in both athlete groups than controls, suggesting that long bones differ in their response to amenorrhea from bones in the trunk (vertebrae, ribs and sternum). Similarly, to eumenorrheic athletes, the amenorrheic athletes had a larger and stronger tibia and femur than controls. As the tibia carries the majority of the body's weight, this indicated that the bone response to regular loading is not attenuated by amenorrhea.

Pysanki's study may provide a suggestion as to why the results of this research study indicate a statistically significant difference in BMD Z scores between the amenorrheic and eumenorrheic groups, yet the amenorrheic group still had a BMD within the normal range. Piaseki's study, alongside this research study, highlight the impact of menstrual status on BMD. They both suggest that the implications of menstrual disturbances were masked by the bone's response and strengthening to the loading activity of exercise, namely long-distance running.

Both studies exemplify the concept of menstrual disturbances as a continuum and suggest that future studies are required to investigate the point at which the benefits of bone remodelling from exercise are hindered by the duration of the menstrual disturbance. This is because it is possible that the loading effects of exercise on BMD will not attenuate the impact of amenorrhea on bone remodelling indefinitely. Therefore, it is likely that having amenorrhea for a certain length of time will eventually lead to an unfavourable skeletal profile in athletes.

Statistically significant results were only observed between the amenorrheic and eumenorrheic groups, and not the oligomenorrheic group. This may be due to the limitation in sample size and equally may also reflect the notion of menstrual disturbances on a continuum to a further extent, as the severity and potentially the time in which an athlete has been oligomenorrheic may impact their BMD. Likewise, when the oligomenorrheic was grouped with the amenorrheic group to form menstrual disturbances and compared to the control, no statistically significant results were found.

Lumbar Spine BMD

Contrastingly to total body BMD, in the study previously mentioned (Piaseki et al., 2018) the torso, lumbar spine, rib, and hips of amenorrheic athletes had a lower BMD than those of the eumenorrheic athletes and controls. Although, it is important to note that this study didn't incorporate separate hip and spine scans. However, this finding suggested to be due to these specific bones not being loaded during running as a result of impact damping, as these upper body bones minimally absorbed the impact of each foot fall and were limited in their direct contribution of the surrounding muscles to locomotion. Therefore, it was argued that the detrimental impact of amenorrhea on these bones is not compensated by the osteogenic effect of increased loading through exercise. Noticeably in this research study, BMD z-score was lower in the lumbar spine for all three groups. As all amenorrheic athletes in this research study had running as their primary sport, this may explain why the mean lumbar spine BMD Z-score of the amenorrheic group was in the athletic risk category (<-1.0) for osteopenia. The Z-score may be in this category as the benefits of loading exercise aren't experienced in this region of the body, despite the total body BMD Z score being within the normal range.

Additionally, there was a significant difference in the anterior-posterior lumbar spine (L1-L4) Z-score between the amenorrheic and eumenorrheic athlete groups. Therefore, amenorrheic athletes have a lower spine BMD Z-score than eumenorrheic athletes at a 95% confidence interval. . The spine is made up of trabecular bone and trabecular loss is more rapid than cortical bone loss. Therefore, the use of DXA scans at the spine confirm this detrimental effect of hypoestrogenism on trabecular bone. However, again there was no difference between the menstrual disturbances and control group, and this may be as a result of the small sample size. Therefore, the lumbar spine is a key area to be monitored, as it is rich in trabecular bone and submitted to little or no weight-bearing during exercise (Young et al., 1994), unless specifically targeted.

Trabecular bone resides within the medullary cavity of the ends of tubular bones and vertebral bodies. It is an open-celled porous cancellous network constituted as 30% mineralized matrix volume and 70% void volume. It has a high surface area/bone matrix volume and so provides a large area facilitating the initiation of bone remodelling. This is a liability when remodelling becomes unbalanced and accelerated as the negative balance results in thinning, and if resorption is deep, results in perforation of the plates (Roelofs et al.,

2012). This liability may offer an explanation as to why there was a strong, but not perfect positive correlation between the total body BMD and the anterior posterior lumbar spine BMD, as trabecular bone only makes up 20% of total bone in the body, suggesting that the lumbar spine is faster to deteriorate than cortical bone, which makes up the majority of the body and therefore influences the total body BMD score. This may also be suggestive of why the lumbar spine BMD isn't perfectly correlated with other BMD variables, as this decreases at a faster rate, for example, there is more metabolically active bone at the spine rather than at the hip. This is because the hip is made up of a combination of trabecular and cortical bone, as opposed to trabecular bone alone.

Femoral Neck and Total Hip

In a recent study of hip geometry using DXA in athletes , it was demonstrated that the cross-sectional area of the femoral neck and shaft was significantly smaller in oligomenorrheic and amenorrheic athletes compared with eumenorrheic athletes however it was similar to that observed in the non-athletes' group (Ackerman et al., 2013, Duckham et al., 2013). This continues to exemplify the benefits of loading on bone health in exercise, as it can often attenuate the impact of menstrual disturbances on bone turnover up until a point. Other studies have observed no difference in femoral neck cross-sectional area between amenorrheic and eumenorrheic athletes. This coincides with the findings of this study, as there was no statistically significant difference between any of the groups' results for femoral neck and total hip. Currently there are inconsistencies in findings for bone strength at the hip as a result of menstrual disturbances. This demonstrates the lack of knowledge and understanding present on menstrual status as a continuum, and whether set markers such as time and severity can be defined for a clearer perspective.

In a 2007 study (Nichols et al.), athletes were grouped according to the type of mechanical loading conferred by their sport. Regularly menstruating athletes participating in high and odd impact sports (track sprinters and field events, soccer, softball, volleyball, tennis, and lacrosse) demonstrated significantly greater hip BMD compared with repetitive (endurance running) and nonimpact (swimmers) athletes and highlighted the strong influence of sport loading modality on the BMD of athletes.

The largest differences were observed when loading modality and menstrual status were combined. Athletes participating in the most osteogenic activities and presenting with the most regular menstrual cycles demonstrate greater bone mass than athletes who participate in less osteogenic sports and present with irregular or absent menstrual cycles. The athletes in the amenorrheic group of this research study had endurance running as their primary sport, as opposed to cycling or swimming. This may suggest why the total hip and femoral neck BMD was not in the risk category for osteopenia, as the benefits of repetitive exercise loading at the hip attenuated the detrimental effects of menstrual disturbances on BMD. However, it is possible that this would have been more noticeable in athletes of high and odd impact sports, as opposed to repetitive.

5.2 Total Body Water

Early studies have described a negative water balance during the first 4 to 5 days of energy deprivation, which then diminishes rapidly. If this energy deprivation continues, the rate of water loss is reduced markedly and water is conserved (Drenick, 1980). It is now well known that, in numerous patients, malnutrition is associated with abnormalities of sodium-potassium pumps, inducing an increase in sodium and water retention (Allison, 2004) and alters the usual proportions of intracellular and extracellular water distributions. One study by Rigaud et al. (2010) suggested that an increase in body extracellular water frequently occurs in anorexic patients under a BMI of 15–16 kg/m^2. This moderated inflation in extracellular water disappears thereafter, during refeeding. The amenorrheic athletes in this research study had an average BMI of 17.65kg/m2, and therefore below 18.5kg/m2, the lowest BMI on the boundary of healthy. However, the amenorrheic athletes did not have a BMI quite as low as those in Rigaud's study. This may suggest why they had body water percentages at the top end of the normal range but were not statistically significant to the eumenorrheic group.

As discussed in the literature review, menstrual disturbances are distributed along a continuum, which incorporates the severity of the menstrual disturbance i.e., luteal phase defects, oligomenorrhea or amenorrhea and the duration of which the menstrual disturbance has lasted. The amenorrheic group in this study had 0-2 periods in the last 12 months, however no data was taken to determine the date of the last period, so the duration of

amenorrhea in this group is unknown but can be estimated to be a minimum of 10-12 months. Therefore, this would be an area of improvement for this study, as this information would be valuable when viewing menstrual disturbances as a continuum.

This study also showed that body water percentage was strongly, negatively correlated with waist:height ratio, highlighting that as waist:height ratio decreases, body water percentage increases, and further demonstrating that amenorrheic athletes have a lower waist:height ratio and a higher body water percentage. This is most likely due to the decrease in overall body mass, indicated by a lower waist:height ratio, which inversely correlates to the overall percentage of body water as a proportion of total body mass, increasing.

This difference in body water percentage between the amenorrheic and eumenorrheic group may have been more apparent and statistically significant had there been a larger sample size and if amenorrhea could be further subdivided into two groups dependent on the duration of this menstrual disturbance. Additionally, earlier research with BIA has shown significant differences in body mass and total body water measurements between the late follicular and mid-luteal phases (Tomazo-Ravnik and Jakopič, 2006), therefore this is another factor that in future studies, needs to be taken into account as this may affect particularly the results of the eumenorrheic group.

Additionally, this study showed that body water percentage was moderately, negatively correlated with BMD total body (Z-score). Therefore, a lower BMD Z-score was associated with a higher body water percentage. A low BMD Z-score is often associated with hypoestrogemia, a decrease in endogenous oestrogen, which causes a disruption of calcium deposition into the bone (Elliot-Sale et al., 2018) and the greater the period of time a woman has been amenorrheic is often indicative of the severity of their low BMD score (Jagielska et al., 2017). This indicates that although body water percentage doesn't cause this disruption of bone density, or vice versa, the correlation between the two highlights that body water percentage is an important variable that is impacted due to menstrual disturbances.

5.3 Waist:Height Ratio

There was a significant difference between the waist:height ratio of the amenorrheic and eumenorrheic group, and as this ratio is used to demonstrate fat distribution and risk for obesity, this difference is suggesting that the amenorrheic group had a significantly lower

body mass and body fat for their height. This implies that amenorrhea can be associated with low body mass, again reinforced through the mean BMI of 17.65 in this group. Although a low body mass can be associated with amenorrhea, the range of BMI in this group (16.3 – 18.6) is indicative that having a BMI classified as underweight is not a prerequisite for menstrual disturbances. This is further exemplified in the oligomenorrheic group, who had BMI ranging from 18.2-23.1, with the majority of the group having a BMI classified as healthy, yet experiencing menstrual cycle disturbances.

Waist:height ratio was also positively correlated with BMD total body, and BMD total body was also positively correlated with BMI and moderately, positively with percentage body fat. If an athlete is subject to LEA, they would be in a calorie deficit and therefore weight loss would come alongside this, suggesting why these different variables are correlated. However, as previously reinforced, having a low body mass or being underweight are not prerequisites for menstrual disturbances.

5.4 Study Considerations

One key limitation to this study is that this is not a longitudinal study and data are collected as one-off measurements rather than multiple measurements being reviewed over an extended period of time. Therefore, as we only took these measurements once, and each athlete will have a different severity of RED-S, dependent on when they started to have menstrual disturbances, we will only see their physical profile at one point in time. This can be limiting to the study as it may make the participants' data less comparable to one another, as menstrual disturbances, alongside their implications, are positioned along a spectrum. This results in athletes with more severe menstrual disturbances that have lasted significantly longer, likely having a more unfavourable bone and cardiometabolic profile than those who have only more recently experienced menstrual disturbances. In addition, another study limitation is that the sample size is relatively small, making it potentially difficult to draw completely reliable and significant conclusions.

Chapter 6: Conclusions and Future Directions

This study confirms the findings of previous studies, that exemplify the differences in bone health in amenorrheic in comparison to eumenorrheic female endurance athletes. The study reinforces that there is often a significant difference in the bone health of these two groups, with total body BMD and lumbar spine (L1-4) Z-score being significantly lower in the amenorrheic athletes v eumenorrheic athletes. However, the benefits of loading and impact-based exercise, such as running, can attenuate the risk posed by menstrual disturbances to bone remodelling, but only to an extent. Future studies should be longitudinal, with larger sample sizes to assess and better understand the point at which the time the menstrual disturbance has lasted for and the severity of it outweigh the benefits brought about from exercise and are detrimental to bone health.

Menstrual disturbances are also thought to have cardiometabolic health implications, and a protocol for this study was in place to investigate that. However, due to COVID-19 restrictions, there was an inability to carry out blood tests and have measurements for glucose, lipoproteins, triglycerides and cholesterol. This led to a lack of understanding and research on cardiometabolic impacts of menstrual disturbances in this study. Furthermore, there is a lack of longitudinal studies and therefore the long-term cardiovascular consequences that amenorrheic athletes face. It has been confirmed that the oestrogen E2 has a cardio-protective role, and therefore hypoestrogenism, which is the manifestation and accumulation of the final consequences of complex hormonal changes from FHA, would result in cardiovascular disease risk factors. Therefore, the independent and combined effects of chronic hypoestrogenism and exercise, together with subclinical dietary behaviours typically observed in amenorrheic athletes, warrants closer examination (O'Donnell and De Souza, 2012).

Chapter 7: Appendix

Table 9: Shapiro Wilks Test for Normality for all body composition outcome variables in the Menstrual Disturbances and Control group

Category	Athlete Menstrual Status	df	Sig.
Body Water Percentage	control	5	.905
	Menstrual disturbance	7	.447
Percentage Body Fat	Control	5	.152
	Menstrual Disturbance	7	.464
Android:Gynoid Ratio	Control	5	.446
	Menstrual Disturbance	7	.537
Waist Circumference	Control	5	.074
	Menstrual Disturbance	7	.471
Waist:Height Ratio	Control	5	.806
	Menstrual Disturbance	7	.141
Waist:Hip Ratio	Control	5	.605
	Menstrual Disturbance	7	.897
BMI	Control	5	.295
	Menstrual Disturbance	7	.390

Table 10: Shapiro Wilks Test for Normality for all bone density outcome variables in the Menstrual Disturbances and Control group

Category	Athlete Menstrual Status	df	Sig.
Total Body BMD (Z-Score)	control	5	.957
	Menstrual disturbance	7	.151
Total Hip (Z-Score)	Control	5	.200
	Menstrual Disturbance	7	.700
Anterior-Posterior Lumbar Spine L1-L4	Control	5	.651
	Menstrual Disturbance	7	.521
Femoral Neck	Control	5	.266
	Menstrual Disturbance	7	.500

Table 11: Shapiro Wilks Test for Normality for all body composition outcome variables in the Amenorrheic, Oligomenorrheic and Control group

Category	Athlete Menstrual Status	df	Sig.
Body Water Percentage	Amenorrhea	4	.068
	Eumenorrhea	5	.944
	Oligomenorrhea	3	.450
Percentage Body Fat	Amenorrhea	4	.291
	Eumenorrhea	5	.152
	Oligomenorrhea	3	.369
Android:Gynoid Ratio	Amenorrhea	4	598
	Eumenorrhea	5	.446
	Oligomenorrhea	3	.938
Waist Circumference	Amenorrhea	4	.459
	Eumenorrhea	5	.074
	Oligomenorrhea	3	702
Waist:Height Ratio	Amenorrhea	4	.104
	Eumenorrhea	5	.806
	Oligomenorrhea	3	.242
Waist:Hip Ratio	Amenorrhea	4	.577
	Eumenorrhea	5	.605
	Oligomenorrhea	3	.780
BMI	Amenorrhea	4	.611
	Eumenorrhea	5	.295
	Oligomenorrhea	3	.933

Table 12: Shapiro Wilks Test for Normality for all bone density outcome variables in the Amenorrheic, Oligomenorrheic and Control group

Category	Athlete Menstrual Status	df	Sig.
Total Body BMD (Z-Score)	Amenorrhea	4	.642
	Eumenorrhea	5	.595
	Oligomenorrhea	3	.685
Total Hip (Z-Score)	Amenorrhea	4	.842
	Eumenorrhea	5	.200
	Oligomenorrhea	3	.085
Anterior-Posterior Lumbar Spine L1-L4	Amenorrhea	4	.555
	Eumenorrhea	5	.651
	Oligomenorrhea	3	.664
Femoral Neck	Amenorrhea	4	.973
	Eumenorrhea	5	.266
	Oligomenorrhea	3	.174

Chapter 8: References

1. Ackerman, K.E. and Misra, M., 2011. Bone health and the female athlete triad in adolescent athletes. *The Physician and sportsmedicine*, *39*(1), pp.131-141.
2. Ackerman, K.E., Pierce, L., Guereca, G., Slattery, M., Lee, H., Goldstein, M. and Misra, M., 2013. Hip structural analysis in adolescent and young adult oligo-amenorrheic and eumenorrheic athletes and nonathletes. *The Journal of Clinical Endocrinology & Metabolism*, *98*(4), pp.1742-1749.
3. Ackerman, K.E., Holtzman, B., Cooper, K.M., Flynn, E.F., Bruinvels, G., Tenforde, A.S., Popp, K.L., Simpkin, A.J. and Parziale, A.L., 2019. Low energy availability surrogates correlate with health and performance consequences of Relative Energy Deficiency in Sport. *British journal of sports medicine*, *53*(10), pp.628-633.
4. Akahoshi, M., Soda, M., Nakashima, E., Tsuruta, M., Ichimaru, S., Seto, S. and Yano, K., 2001. Effects of age at menopause on serum cholesterol, body mass index, and blood pressure. *Atherosclerosis*, *156*(1), pp.157-163.
5. Allaway, H.C., Southmayd, E.A. and De Souza, M.J., 2016. The physiology of functional hypothalamic amenorrhea associated with energy deficiency in exercising women and in women with anorexia nervosa. *Hormone Molecular Biology and Clinical Investigation*, *25*(2), pp.91-119.
6. Allison, S.P. and Lobo, D.N., 2004. Fluid and electrolytes in the elderly. *Current Opinion in Clinical Nutrition & Metabolic Care*, *7*(1), pp.27-33.
7. American Academy of Pediatrics and American College of Obstetricians and Gynecologists, 2006. Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Pediatrics*, *118*(5), pp.2245-2250.
8. Ayres, S., Baer, J. and Subbiah, M.R., 1998. Exercised-induced increase in lipid peroxidation parameters in amenorrhelc female athletes. *Fertility and sterility*, *69*(1), pp.73-77.
9. Bacchi, E., Spiazzi, G., Zendrini, G., Bonin, C. and Moghetti, P., 2013. Low body weight and menstrual dysfunction are common findings in both elite and amateur ballet dancers. *Journal of endocrinological investigation*, *36*(5), pp.343-346.
10. Baer, J.T., 1999. Lipid status in female athletes with lower estrogen: Applications to cardiovascular risk. *American Journal of Health Studies*, *15*(1), p.29.

11. Bangsbo, J., Graham, T.E., Kiens, B. and Saltin, B., 1992. Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *The Journal of Physiology*, *451*(1), pp.205-227.
12. Barreiro, M.L. and Tena-Sempere, M., 2004. Ghrelin and reproduction: a novel signal linking energy status and fertility?. *Molecular and cellular endocrinology*, *226*(1-2), pp.1-9.
13. Berga, S.L., Mortola, J.F., Girton, L., Suh, B., Laughlin, G., Pham, P. and Yen, S.S.C., 1989. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. The Journal of Clinical Endocrinology & Metabolism, 68(2), pp.301-308.
14. Bonnefont-Rousselot, D., Thérond, P., Beaudeux, J.L., Peynet, J., Le-grand, A. and Delattre, J., 1999. High density lipoproteins (HDL) and the oxidative hypothesis of atherosclerosis.
15. Budak, E., Sánchez, M.F., Bellver, J., Cerveró, A., Simón, C. and Pellicer, A., 2006. Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3-36 with the reproductive system. *Fertility and sterility*, *85*(6), pp.1563-1581.
16. Burger, A.G., Berger, M., Wimpfheimer, K. and Danforth, E., 1980. Interrelationships between energy metabolism and thyroid hormone metabolism during starvation in the rat. *European Journal of Endocrinology*, *93*(3), pp.322-331.
17. Carlberg, K.A., Buckman, M.T., Peake, G.T. and Riedesel, M.L., 1983. A survey of menstrual function in athletes. *European Journal of Applied Physiology and Occupational Physiology*, *51*(2), pp.211-222.
18. Carro, E., Pinilla, L., Seoane, L.M., Considine, R.V., Aguilar, E., Casanueva, F.F. and Dieguez, C., 1997. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. *Neuroendocrinology*, *66*(6), pp.375-377.
19. Caprio, M., Fabbrini, E., Isidori, A.M., Aversa, A. and Fabbri, A., 2001. Leptin in reproduction. *Trends in Endocrinology & Metabolism*, *12*(2), pp.65-72.
20. Castellano, J.M., Navarro, V.M., Fernandez-Fernandez, R., Nogueiras, R., Tovar, S., Roa, J., Vazquez, M.J., Vigo, E., Casanueva, F.F., Aguilar, E. and Pinilla, L., 2005. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology*, *146*(9), pp.3917-3925.

21. Castellano, J.M. and Tena-Sempere, M., 2013. Metabolic regulation of kisspeptin. *Kisspeptin signaling in reproductive biology*, pp.363-383.
22. Chan, J.L. and Mantzoros, C.S., 2005. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *The lancet*, *366*(9479), pp.74-85.
23. Chan, Y.M., Butler, J.P., Sidhoum, V.F., Pinnell, N.E. and Seminara, S.B., 2012. Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. *The Journal of Clinical Endocrinology & Metabolism*, *97*(8), pp.E1458-E1467.
24. Clark, L.R., Dellogono, M.J., Mangano, K.M. and Wilson, T.A., 2018. Clinical menstrual dysfunction is associated with low energy availability but not dyslipidemia in division I female endurance runners. *Journal of Exercise Physiology Online*, *21*(2), pp.265-277.
25. Cunningham, M.J., Clifton, D.K. and Steiner, R.A., 1999. Leptin's actions on the reproductive axis: perspectives and mechanisms. *Biology of reproduction*, *60*(2), pp.216-222.
26. Danforth Jr, E. and Burger, A.G., 1989. The impact of nutrition on thyroid hormone physiology and action. *Annual review of nutrition*, *9*, pp.201-227.
27. Darmaun, D., Cirillo, D., Koziet, J., Chauvet, D., Young, V.R. and Robert, J.J., 1988. Whole body glucose kinetics in type I diabetes studied with [6, 6-2H] and [U-13C]-glucose and the artificial B-cell. *Metabolism*, *37*(5), pp.491-498.
28. De Souza, M.J. and Williams, N.I., 2004. Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Human reproduction update*, *10*(5), pp.433-448.
29. De Souza, M.J., Lee, D.K., VanHeest, J.L., Scheid, J.L., West, S.L. and Williams, N.I., 2007. Severity of energy-related menstrual disturbances increases in proportion to indices of energy conservation in exercising women. *Fertility and sterility*, *88*(4), pp.971-975.
30. De Souza, M.J., Toombs, R.J., Scheid, J.L., O'Donnell, E., West, S.L. and Williams, N.I., 2010. High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures. *Human reproduction*, *25*(2), pp.491-503.

31. De Souza, M.J., Nattiv, A., Joy, E., Misra, M., Williams, N.I., Mallinson, R.J., Gibbs, J.C., Olmsted, M., Goolsby, M., Matheson, G. and Panel, E., 2014. 2014 Female Athlete Triad Coalition Consensus Statement on treatment and return to play of the female athlete triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. *British journal of sports medicine*, *48*(4), pp.289-289.
32. De Souza, M.J., Koltun, K.J., Etter, C.V. and Southmayd, E.A., 2017. Current status of the female athlete triad: update and future directions. *Current osteoporosis reports*, *15*(6), pp.577-587.
33. De Souza, M.J., Koltun, K.J. and Williams, N.I., 2019. The role of energy availability in reproductive function in the female athlete triad and extension of its effects to men: an initial working model of a similar syndrome in male athletes. *Sports Medicine*, *49*(2), pp.125-137.
34. Dhillo, W.S., Chaudhri, O.B., Thompson, E.L., Murphy, K.G., Patterson, M., Ramachandran, R., Nijher, G.K., Amber, V., Kokkinos, A., Donaldson, M. and Ghatei, M.A., 2007. Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *The Journal of Clinical Endocrinology & Metabolism*, *92*(10), pp.3958-3966.
35. Dhillon, S.S. and Belsham, D.D., 2011. Leptin differentially regulates NPY secretion in hypothalamic cell lines through distinct intracellular signal transduction pathways. *Regulatory peptides*, *167*(2-3), pp.192-200.
36. Dietschy, J.M., 1997. Theoretical considerations of what regulates low-density-lipoprotein and high-density-lipoprotein cholesterol. *The American journal of clinical nutrition*, *65*(5), pp.1581S-1589S.
37. DiPietro, L. and Stachenfeld, N.S., 2006. The myth of the female athlete triad. *British journal of sports medicine*, *40*(6), pp.490-493.Drenick, E.J., 1980. The effects of acute and prolonged fasting and refeeding on water, electrolyte, and acid-base metabolism. *Clinical Disorders of Fluid and Electrolyte Metabolism*, pp.1481-1501.
38. Drew, M.K., Vlahovich, N., Hughes, D., Appaneal, R., Peterson, K., Burke, L., Lundy, B., Toomey, M., Watts, D., Lovell, G. and Praet, S., 2017. A multifactorial evaluation of illness risk factors in athletes preparing for the Summer Olympic Games. *Journal of Science and Medicine in Sport*, *20*(8), pp.745-750.

39. Dipla, K., Kraemer, R.R., Constantini, N.W. and Hackney, A.C., 2020. Relative energy deficiency in sports (RED-S): Elucidation of endocrine changes affecting the health of males and females. *Hormones*, pp.1-13.
40. Duckham, R.L., Peirce, N., Bailey, C.A., Summers, G., Cameron, N. and Brooke-Wavell, K., 2013. Bone geometry according to menstrual function in female endurance athletes. *Calcified tissue international*, *92*(5), pp.444-450.
41. Dušek, T., 2001. Influence of high intensity training on menstrual cycle disorders in athletes. *Croat Med J*, *42*(1), pp.79-82.
42. Edwards, J.E., Lindeman, A.K., Mikesky, A.E. and Stager, J.M., 1993. Energy balance in highly trained female endurance runners. *Medicine and science in sports and exercise*, *25*(12), pp.1398-1404.
43. Elliott-Sale, K.J., Tenforde, A.S., Parziale, A.L., Holtzman, B. and Ackerman, K.E., 2018. Endocrine effects of relative energy deficiency in sport. *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(4), pp.335-349.
44. Enns, D.L. and Tiidus, P.M., 2010. The influence of estrogen on skeletal muscle. *Sports medicine*, *40*(1), pp.41-58.
45. Feillet, F., Feillet-Coudray, C., Bard, J.M., Parra, H.J., Favre, E., Kabuth, B., Fruchart, J.C. and Vidailhet, M., 2000. Plasma cholesterol and endogenous cholesterol synthesis during refeeding in anorexia nervosa. *Clinica chimica acta*, *294*(1-2), pp.45-56.
46. Feingold, K.R. and Grunfeld, C., 2012. Lipids: a key player in the battle between the host and microorganisms1. *Journal of lipid research*, *53*(12), pp.2487-2489.
47. Feng, X. and McDonald, J.M., 2011. Disorders of bone remodeling. *Annual Review of Pathology: Mechanisms of Disease*, *6*, pp.121-145.
48. Fernández-Fernández, R., Tena-Sempere, M., Aguilar, E. and Pinilla, L., 2004. Ghrelin effects on gonadotropin secretion in male and female rats. *Neuroscience letters*, *362*(2), pp.103-107.
49. Friday KE, Drinkwater BL, Bruemmer B, Chesnut 3rd C, Chait A 1993 Elevated plasma low-density lipoprotein and high-density lipoprotein cholesterol levels in amenorrheic athletes: effects of endogenous hormone status and nutrient intake. J Clin Endocrinol Metab 77:1605–1609

50. Friedewald, W.T., 1972. FRIEDEWALD, WT, LEVY, RI AND FREDRICKSON, DS ESTIMATION OF THE CONCENTRATION OF LOW-DENSITY LIPOPROTEIN CHOLESTEROL IN PLASMA, WITHOUT USE OF THE PREPARATIVE ULTRACENTRIFUGE. Clinical Chemistry, 18, pp.499-502.
51. Genazzani, A.D., Gastaldi, M., Volpe, A., Petraglia, F. and Genazzani, A.R., 1995. Spontaneous episodic release of adenohypophyseal hormones in hypothalamic amenorrhea. *Gynecological Endocrinology*, *9*(4), pp.325-334.
52. George, J.T., Anderson, R.A. and Millar, R.P., 2012. Kisspeptin-10 stimulation of gonadotrophin secretion in women is modulated by sex steroid feedback. *Human reproduction*, *27*(12), pp.3552-3559.
53. Gejl, K.D., Hvid, L.G., Frandsen, U., Jensen, K., Sahlin, K. and Ørtenblad, N., 2014. Muscle glycogen content modifies SR Ca2+ release rate in elite endurance athletes. *Medicine & Science in Sports & Exercise*, *46*(3), pp.496-505.
54. Gibbs, J.C., Williams, N.I. and De Souza, M.J., 2013. Prevalence of individual and combined components of the female athlete triad. *Medicine & Science in Sports & Exercise*, *45*(5), pp.985-996.
55. Gibbs, J.C., Williams, N.I., Scheid, J.L., Toombs, R.J. and De Souza, M.J., 2011. The association of a high drive for thinness with energy deficiency and severe menstrual disturbances: confirmation in a large population of exercising women. *International journal of sport nutrition and exercise metabolism*, *21*(4), pp.280-290.
56. Golden, N.H. and Carlson, J.L., 2008. The pathophysiology of amenorrhea in the adolescent. *Annals of the New York Academy of Sciences*, *1135*(1), pp.163-178.
57. Gordon, C.M., 2010. Functional hypothalamic amenorrhea. *New England Journal of Medicine*, *363*(4), pp.365-371.
58. Gordon, C.M., Ackerman, K.E., Berga, S.L., Kaplan, J.R., Mastorakos, G., Misra, M., Murad, M.H., Santoro, N.F. and Warren, M.P., 2017. Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism*, *102*(5), pp.1413-1439.
59. Guetta, V. and Cannon III, R.O., 1996. Cardiovascular effects of estrogen and lipid-lowering therapies in postmenopausal women. *Circulation*, *93*(10), pp.1928-1937.

60. Hama, H., Saito, A., Takeda, T., Tanuma, A., Xie, Y., Sato, K., Kazama, J.J. and Gejyo, F., 2004. Evidence indicating that renal tubular metabolism of leptin is mediated by megalin but not by the leptin receptors. *Endocrinology*, *145*(8), pp.3935-3940.
61. Hamm, M.L., Bhat, G.K., Thompson, W.E. and Mann, D.R., 2004. Folliculogenesis is impaired and granulosa cell apoptosis is increased in leptin-deficient mice. *Biology of reproduction*, *71*(1), pp.66-72.
62. Harber, V.J., 2004. Energy balance and reproductive function in active women. *Canadian journal of applied physiology*, *29*(1), pp.48-58.
63. Helge, E.W. and Kanstrup, I.L., 2002. Bone density in female elite gymnasts: impact of muscle strength and sex hormones. *Medicine and science in sports and exercise*, *34*(1), pp.174-180.
64. Hill, J.W., Elmquist, J.K. and Elias, C.F., 2008. Hypothalamic pathways linking energy balance and reproduction. *American Journal of Physiology-Endocrinology and Metabolism*, *294*(5), pp.E827-E832.
65. Hilton, L.K. and Loucks, A.B., 2000. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. *American Journal of Physiology-Endocrinology And Metabolism*, *278*(1), pp.E43-E49.
66. Hoch, A.Z., Pajewski, N.M., Moraski, L., Carrera, G.F., Wilson, C.R., Hoffmann, R.G., Schimke, J.E. and Gutterman, D.D., 2009. Prevalence of the female athlete triad in high school athletes and sedentary students. *Clinical journal of sport medicine: official journal of the Canadian Academy of Sport Medicine*, *19*(5), p.421.
67. Homma, Y., Homma, K., Iizuka, S. and Iigaya, K., 2002. A case of anorexia nervosa with severe hyperlipoproteinemia. *International Journal of Eating Disorders*, *32*(1), pp.121-124.
68. Hutson, M.J., O'Donnell, E., Brooke-Wavell, K., Sale, C. and Blagrove, R.C., 2020. Effects of low energy availability on bone health in endurance athletes and high-impact exercise as a potential countermeasure: a narrative review. *Sports Medicine*, pp.1-13.
69. Ihle, R. and Loucks, A.B., 2004. Dose-response relationships between energy availability and bone turnover in young exercising women. *Journal of bone and mineral research*, *19*(8), pp.1231-1240.

70. International Atomic Energy Agency https://www.iaea.org/Publications/Factsheets/English/radlife
71. Jayasena, C.N., Nijher, G.M., Chaudhri, O.B., Murphy, K.G., Ranger, A., Lim, A., Patel, D., Mehta, A., Todd, C., Ramachandran, R. and Salem, V., 2009. Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. *The Journal of Clinical Endocrinology & Metabolism*, *94*(11), pp.4315-4323.
72. Jayasena, C.N., Nijher, G.M.K., Abbara, A., Murphy, K.G., Lim, A., Patel, D., Mehta, A., Todd, C., Donaldson, M., Trew, G.H. and Ghatei, M.A., 2010. Twice-weekly administration of kisspeptin-54 for 8 weeks stimulates release of reproductive hormones in women with hypothalamic amenorrhea. *Clinical Pharmacology & Therapeutics*, *88*(6), pp.840-847.
73. Jayasena, C.N., Comninos, A.N., Nijher, G.M.K., Abbara, A., De Silva, A., Veldhuis, J.D., Ratnasabapathy, R., Izzi-Engbeaya, C., Lim, A., Patel, D.A. and Ghatei, M.A., 2013. Twice-daily subcutaneous injection of kisspeptin-54 does not abolish menstrual cyclicity in healthy female volunteers. *The Journal of Clinical Endocrinology & Metabolism*, *98*(11), pp.4464-4474.
74. Jayasena, C.N., Abbara, A., Veldhuis, J.D., Comninos, A.N., Ratnasabapathy, R., De Silva, A., Nijher, G.M., Ganiyu-Dada, Z., Mehta, A., Todd, C. and Ghatei, M.A., 2014. Increasing LH pulsatility in women with hypothalamic amenorrhoea using intravenous infusion of Kisspeptin-54. *The Journal of Clinical Endocrinology & Metabolism*, *99*(6), pp.E953-E961.
75. Johnston, C.C. and Slemenda, C.W., 1994. Peak bone mass, bone loss and risk of fracture. *Osteoporosis international*, *4*(1), pp.S43-S45.
76. Joy, E., Kussman, A. and Nattiv, A., 2016. 2016 update on eating disorders in athletes: A comprehensive narrative review with a focus on clinical assessment and management. *British journal of sports medicine*, *50*(3), pp.154-162.
77. Kaiserauer, S.U.S.A.N.N.E., Snyder, A.C., Sleeper, M.A.R.K. and Zierath, J.U.L.E.E.N., 1989. Nutritional, physiological, and menstrual status of distance runners. *Medicine and Science in Sports and Exercise*, *21*(2), pp.120-125.

78. Kanis, J.A., Johnell, O., Oden, A., Dawson, A., De Laet, C. and Jonsson, B.J.O.I., 2001. Ten year probabilities of osteoporotic fractures according to BMD and diagnostic thresholds. *Osteoporosis International*, *12*(12), pp.989-995.
79. Keay, N., Overseas, A. and Francis, G., 2020. Awareness and indicators of low energy availability in male and female dancers. *medRxiv*.
80. Khosla, S., Oursler, M.J. and Monroe, D.G., 2012. Estrogen and the skeleton. *Trends in Endocrinology & Metabolism*, *23*(11), pp.576-581.
81. Koehler, K., Hoerner, N.R., Gibbs, J.C., Zinner, C., Braun, H., De Souza, M.J. and Schaenzer, W., 2016. Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones. *Journal of sports sciences*, *34*(20), pp.1921-1929.
82. Koltun, K.J., De Souza, M.J., Scheid, J.L. and Williams, N.I., 2020. Energy availability is associated with luteinizing hormone pulse frequency and induction of luteal phase defects. *The Journal of Clinical Endocrinology & Metabolism*, *105*(1), pp.185-193.
83. Korsten-Rech. U. FIMS Position Statement 2011. The female athlete triad. Int SportMed J 2011; 12: 156-9.
84. Krentz, E.M. and Warschburger, P., 2011. Sports-related correlates of disordered eating in aesthetic sports. *Psychology of Sport and Exercise*, *12*(4), pp.375-382.
85. Krentz, E.M. and Warschburger, P., 2013. A longitudinal investigation of sports-related risk factors for disordered eating in aesthetic sports. *Scandinavian journal of medicine & science in sports*, *23*(3), pp.303-310.
86. Lania, A., Gianotti, L., Gagliardi, I., Bondanelli, M., Vena, W. and Ambrosio, M.R., 2019. Functional hypothalamic and drug-induced amenorrhea: an overview. Journal of endocrinological investigation, 42(9), pp.1001-1010.
87. Laughlin, G.A. and Yen, S.S.C., 1997. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. *The Journal of Clinical Endocrinology & Metabolism*, *82*(1), pp.318-321.
88. Lewiecki, E.M., Watts, N.B., McClung, M.R., Petak, S.M., Bachrach, L.K., Shepherd, J.A., Downs Jr, R.W. and International Society for Clinical Densitometry, 2004. Official positions of the international society for clinical densitometry. *The Journal of Clinical Endocrinology & Metabolism*, *89*(8), pp.3651-3655.

89. Lieberman, J.L., De Souza, M.J., Wagstaff, D.A. and Williams, N.I., 2018. Menstrual disruption with exercise is not linked to an energy availability threshold. *Medicine and science in sports and exercise*, *50*(3), p.551.
90. Liscum, L. and Munn, N.J., 1999. Intracellular cholesterol transport. *Biochimica et biophysica acta*, *1438*(1), pp.19-37.
91. Loucks, A.B., Laughlin, G.A., Mortola, J.F., Girton, L., Nelson, J.C. and Yen, S.S., 1992. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. *The Journal of Clinical Endocrinology & Metabolism*, *75*(2), pp.514-518.
92. Loucks, A.B. and Callister, R., 1993. Induction and prevention of low-T3 syndrome in exercising women. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *264*(5), pp.R924-R930.
93. Loucks, A.B. and Heath, E.M., 1994. Induction of low-T3 syndrome in exercising women occurs at a threshold of energy availability. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *266*(3), pp.R817-R823.
94. Loucks, A.B., Verdun, M., Heath, E.M. and (With the Technical Assistance of T. Law, Sr. and JR Thuma), 1998. Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. *Journal of applied physiology*, *84*(1), pp.37-46.
95. Logue, D., Madigan, S.M., Delahunt, E., Heinen, M., Mc Donnell, S.J. and Corish, C.A., 2018. Low energy availability in athletes: a review of prevalence, dietary patterns, physiological health, and sports performance. *Sports Medicine*, *48*(1), pp.73-96.
96. Logue, D.M., Madigan, S.M., Melin, A., Delahunt, E., Heinen, M., Donnell, S.J.M. and Corish, C.A., 2020. Low energy availability in athletes 2020: an updated narrative review of prevalence, risk, within-day energy balance, knowledge, and impact on sports performance. *Nutrients*, *12*(3), p.835.
97. Loucks, A.B., Horvath, S.M. and Freedson, P.S., 1984. Menstrual status and validation of body fat prediction in athletes. *Human biology*, pp.383-392.
98. Loucks, A.B. and Horvath, S.M., 1985. Athletic amenorrhea: a review. *Medicine and science in sports and exercise*, *17*(1), pp.56-72.
99. Loucks, A.B., Laughlin, G.A., Mortola, J.F., Girton, L., Nelson, J.C. and Yen, S.S., 1992. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. *The Journal of Clinical Endocrinology & Metabolism*, *75*(2), pp.514-518.

100. Loucks, A.B. and Thuma, J.R., 2003. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *The Journal of Clinical Endocrinology & Metabolism*, *88*(1), pp.297-311.
101. Loucks, A.B., 2004. Energy balance and body composition in sports and exercise. *Journal of sports sciences*, *22*(1), pp.1-14.
102. Loucks, A.B., 2013. Energy balance and energy availability. *The encyclopaedia of sports medicine: An IOC medical commission publication*, *19*, pp.72-87.
103. Luque, R.M., Kineman, R.D. and Tena-Sempere, M., 2007. Regulation of hypothalamic expression of KiSS-1 and GPR54 genes by metabolic factors: analyses using mouse models and a cell line. *Endocrinology*, *148*(10), pp.4601-4611.
104. MacLaren, D.P.M., Reilly, T., Campbell, I.T. and Hopkin, C., 1999. Hormonal and metabolic responses to maintained hyperglycemia during prolonged exercise. *Journal of Applied Physiology*, *87*(1), pp.124-131.
105. Márquez, S. and Molinero, O., 2013. Energy availability, menstrual dysfunction and bone health in sports; an overview of the female athlete triad. *Nutricion hospitalaria*, *28*(4), pp.1010-1017.
106. Matkovic, V., Jelic, T., Wardlaw, G.M., Ilich, J.Z., Goel, P.K., Wright, J.K., Andon, M.B., Smith, K.T. and Heaney, R.P., 1994. Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. *The Journal of clinical investigation*, *93*(2), pp.799-808.
107. McCarthy, M.M., 2013. A piece in the puzzle of puberty. *Nature neuroscience*, *16*(3), pp.251-253.
108. Meczekalski, B., Podfigurna-Stopa, A., Warenik-Szymankiewicz, A. and Genazzani, A.R., 2008. Functional hypothalamic amenorrhea: current view on neuroendocrine aberrations. *Gynecological endocrinology*, *24*(1), pp.4-11.
109. Meczekalski, B., Katulski, K., Czyzyk, A., Podfigurna-Stopa, A. and Maciejewska-Jeske, M., 2014. Functional hypothalamic amenorrhea and its influence on women's health. *Journal of endocrinological investigation*, *37*(11), pp.1049-1056.
110. Medical Research Council, 2014. MRC ethics series Human Tissue and Biological Samples for Use in Research: Operational and Ethical Guidelines. https://mrc.ukri.org/publications/browse/human-tissue-and-biological-samples-for-use-in-research/

111. Melin, A., Tornberg, Å.B., Skouby, S., Faber, J., Ritz, C., Sjödin, A. and Sundgot-Borgen, J., 2014. The LEAF questionnaire: a screening tool for the identification of female athletes at risk for the female athlete triad. *British Journal of Sports Medicine*, *48*(7), pp.540-545.
112. Melin, A., Tornberg, Å.B., Skouby, S., Møller, S.S., Sundgot-Borgen, J., Faber, J., Sidelmann, J.J., Aziz, M. and Sjödin, A., 2015. Energy availability and the female athlete triad in elite endurance athletes. *Scandinavian journal of medicine & science in sports*, *25*(5), pp.610-622.
113. Mendelsohn, F.A. and Warren, M.P., 2010. Anorexia, bulimia, and the female athlete triad: evaluation and management. *Endocrinology and Metabolism Clinics*, *39*(1), pp.155-167.
114. Melin, A.K., Heikura, I.A., Tenforde, A. and Mountjoy, M., 2019. Energy availability in athletics: health, performance, and physique. *International Journal of Sport Nutrition and Exercise Metabolism*, *29*(2), pp.152-164.
115. Misra, M., Miller, K.K., Kuo, K., Griffin, K., Stewart, V., Hunter, E., Herzog, D.B. and Klibanski, A., 2005. Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. *American Journal of Physiology-Endocrinology and Metabolism*, *289*(2), pp.E347-E356.
116. Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R. and Ljungqvist, A., 2015. The IOC relative energy deficiency in sport clinical assessment tool (RED-S CAT).
117. Noel Bairey Merz, C., Johnson, B.D., Sharaf, B.L., Bittner, V., Berga, S.L., Braunstein, G.D., Hodgson, T.K., Matthews, K.A., Pepine, C.J., Reis, S.E. and Reichek, N., 2003. Hypoestrogenemia of hypothalamic origin and coronary artery disease in premenopausal women: a report from the NHLBI-sponsored WISE study. Journal of the American College of Cardiology, 41(3), pp.413-419.
118. Mikhael, S., Punjala-Patel, A. and Gavrilova-Jordan, L., 2019. Hypothalamic-pituitary-ovarian axis disorders impacting female fertility. *Biomedicines*, *7*(1), p.5.
119. Morghental AP. Female athlete triad. J Chiropr Med 2002; 1: 97-106.

120. Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R. and Ljungqvist, A., 2014. The IOC consensus statement: beyond the female athlete triad—relative energy deficiency in sport (RED-S). *British journal of sports medicine*, *48*(7), pp.491-497.
121. Mountjoy, M. ed., 2014. *Handbook of Sports Medicine and Science, The Female Athlete*. John Wiley & Sons.
122. Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., Meyer, N., Sherman, R., Steffen, K., Budgett, R. and Ljungqvist, A., 2015. Authors' 2015 additions to the IOC consensus statement: Relative Energy Deficiency in Sport (RED-S).
123. Mountjoy, M., Sundgot-Borgen, J., Burke, L., Ackerman, K.E., Blauwet, C., Constantini, N., Lebrun, C., Lundy, B., Melin, A., Meyer, N. and Sherman, R., 2018. International Olympic Committee (IOC) consensus statement on relative energy deficiency in sport (RED-S): 2018 update. International journal of sport nutrition and exercise metabolism, 28(4), pp.316-331.
124. Movaseghi, S., Dadgostar, H., Dahaghin, S., Chimeh, N., Alenabi, T., Dadgostar, E. and Davatchi, F., 2012. Clinical manifestations of the female athlete triad among some Iranian athletes. *Medicine & Science in Sports & Exercise*, *44*(5), pp.958-965.
125. Myburgh, K.H., Hutchins, J., Fataar, A.B., Hough, S.F. and Noakes, T.D., 1990. Low bone density is an etiologic factor for stress fractures in athletes. *Annals of internal medicine*, *113*(10), pp.754-759.
126. Myerson, M.E.R.L.E., Gutin, B.E.R.N.A.R.D., Warren, M.P., May, M.T., Contento, I.S.O.B.E.L., Lee, M.I.C.H.A.E.L., Pi-Sunyer, F.X., Pierson Jr, R.N. and Brooks-Gunn, J., 1991. Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. *Medicine and science in sports and exercise*, *23*(1), pp.15-22.
Nattiv A, Loucks AB, Manore MM, Sanborn CF, Sundgot-Borgen J, Warren MP., 2007. American College of Sports Medicine position stand. The female athlete triad. Med Sci Sports Exerc 39, pp.1867–1882
127. Navarro, V.M. and Kaiser, U.B., 2013. Metabolic influences on neuroendocrine regulation of reproduction. *Current opinion in endocrinology, diabetes, and obesity*, *20*(4), p.335.

128. Nichols, D.L., Bonnick, S.L. and Sanborn, C.F., 2000. Bone health and osteoporosis. *Clinics in sports medicine*, *19*(2), pp.233-249.
129. Nichols, J.F., Rauh, M.J., Lawson, M.J., Ji, M. and Barkai, H.S., 2006. Prevalence of the female athlete triad syndrome among high school athletes. Archives of pediatrics & adolescent medicine, 160(2), pp.137-142.
130. Nichols, D.L., Sanborn, C.F. and Essery, E.V., 2007. Bone density and young athletic women. *Sports Medicine*, *37*(11), pp.1001-1014.
131. Nichols, J.F., Rauh, M.J., Barrack, M.T. and Barkai, H.S., 2007. Bone mineral density in female high school athletes: interactions of menstrual function and type of mechanical loading. *Bone*, *41*(3), pp.371-377.
132. Nordström, A., Karlsson, C., Nyquist, F., Olsson, T., Nordström, P. and Karlsson, M., 2005. Bone loss and fracture risk after reduced physical activity. *Journal of bone and mineral research*, *20*(2), pp.202-207.
133. O'Donnell, E. and De Souza, M.J., 2004. The Cardiovascular Effects of Chronic Hypoestrogenism in Amenorrhoeic Athletes. *Sports Medicine*, *34*(9), pp.601-627.
134. O'Donnell, E., Goodman, J.M. and Harvey, P.J., 2011. Cardiovascular consequences of ovarian disruption: A focus on functional hypothalamic amenorrhea in physically active women. *The Journal of Clinical Endocrinology & Metabolism*, *96*(12), pp.3638-3648.
135. Otis, C.L., 1992. Exercise-associated amenorrhea. *Clinics in sports medicine*, *11*(2), pp.351-362.
136. Piasecki, J., Ireland, A., Piasecki, M., Cameron, J., McPhee, J.S. and Degens, H., 2018. The strength of weight-bearing bones is similar in amenorrheic and eumenorrheic elite long-distance runners. *Scandinavian journal of medicine & science in sports*, *28*(5), pp.1559-1568.
137. Pollock, N., Grogan, C., Perry, M., Pedlar, C., Cooke, K., Morrissey, D. and Dimitriou, L., 2010. Bone-mineral density and other features of the female athlete triad in elite endurance runners: a longitudinal and cross-sectional observational study. *International journal of sport nutrition and exercise metabolism*, *20*(5), pp.418-426.
138. Practice Committee of the American Society for Reproductive Medicine, 2006. Current evaluation of amenorrhea. *Fertility and sterility*, *86*(5), pp.S148-S155.

139. Prior, J.C., Vigna, Y.M., Schechter, M.T. and Burgess, A.E., 1990. Spinal bone loss and ovulatory disturbances. *New England Journal of Medicine*, *323*(18), pp.1221-1227.
140. Raisz, L.G. and Rodan, G.A., 2003. Pathogenesis of osteoporosis. *Endocrinology and metabolism clinics of North America*, *32*(1), pp.15-24.
141. Raj, M.A., Creech, J.A. and Rogol, A.D., 2020. Female athlete triad. *StatPearls [Internet]*.
142. Reed, J.L., De Souza, M.J., Mallinson, R.J., Scheid, J.L. and Williams, N.I., 2015. Energy availability discriminates clinical menstrual status in exercising women. *Journal of the International Society of Sports Nutrition*, *12*(1), pp.1-11.
143. Redman, L.M. and Loucks, A.B., 2005. Menstrual disorders in athletes. *Sports Medicine*, *35*(9), pp.747-755.
144. Rickenlund, A., Eriksson, M.J., Schenck-Gustafsson, K. and Hirschberg, A.L., 2005. Amenorrhea in female athletes is associated with endothelial dysfunction and unfavorable lipid profile. *The Journal of Clinical Endocrinology & Metabolism*, *90*(3), pp.1354-1359.
145. Rigaud, D., Boulier, A., Tallonneau, I., Brindisi, M.C. and Rozen, R., 2010. Body fluid retention and body weight change in anorexia nervosa patients during refeeding. *Clinical Nutrition*, *29*(6), pp.749-755.
146. Riggs, B.L., Khosla, S., Atkinson, E.J., Dunstan, C.R. and Melton, L.J., 2003. Evidence that type I osteoporosis results from enhanced responsiveness of bone to estrogen deficiency. *Osteoporosis international*, *14*(9), pp.728-733.
147. Roberts, R.E., Farahani, L., Webber, L. and Jayasena, C., 2020. Current understanding of hypothalamic amenorrhoea. *Therapeutic Advances in Endocrinology and Metabolism*, *11*, p.2042018820945854.
148. Robinson, T.L., Snow-Harter, C., Taaffe, D.R., Gillis, D., Shaw, J. and Marcus, R., 1995. Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. *Journal of Bone and Mineral Research*, *10*(1), pp.26-35.
149. Rodriguez-Pacheco, F., Martinez-Fuentes, A.J., Tovar, S., Pinilla, L., Tena-Sempere, M., Dieguez, C., Castano, J.P. and Malagon, M.M., 2007. Regulation of pituitary cell function by adiponectin. *Endocrinology*, *148*(1), pp.401-410.

150. Roelofs, A.J., Stewart, C.A., Sun, S., Błażewska, K.M., Kashemirov, B.A., McKenna, C.E., Russell, R.G.G., Rogers, M.J., Lundy, M.W., Ebetino, F.H. and Coxon, F.P., 2012. Influence of bone affinity on the skeletal distribution of fluorescently labeled bisphosphonates in vivo. Journal of Bone and Mineral Research, 27(4), pp.835-847.
151. Roeters van Lennep, J.E., Westerveld, H.T., Erkelens, D.W. and van der Wall, E.E., 2002. Risk factors for coronary heart disease: implications of gender. *Cardiovascular research*, *53*(3), pp.538-549.
152. Rosenbaum, M., Hirsch, J., Murphy, E. and Leibel, R.L., 2000. Effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. *The American journal of clinical nutrition*, *71*(6), pp.1421-1432.
153. Rosenfield, R.L. and Ehrmann, D.A., 2016. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocrine reviews, 37(5), pp.467-520.
154. Roupas, N.D. and Georgopoulos, N.A., 2011. Menstrual function in sports. *Hormones*, *10*(2), pp.104-116.
155. Rosen, 2018. Amenorrhea in the Female Athlete: 8 Myths Debunked. https://www.gaudianiclinic.com/gaudiani-clinic-blog/2018/10/10/amenorrhea-in-the-female-athlete-8-myths-debunked#:~:text=Myth%201%3A%20It%20is%20normal,of%20athletic%20or%20fitness%20training.
156. Russell, M. and Misra, M., 2010. Influence of ghrelin and adipocytokines on bone mineral density in adolescent female athletes with amenorrhea and eumenorrheic athletes. *Cytokines, growth mediators and physical activity in children during puberty*, *55*, pp.103-113.
157. Russell, M., Stark, J., Nayak, S., Miller, K.K., Herzog, D.B., Klibanski, A. and Misra, M., 2009. Peptide YY in adolescent athletes with amenorrhea, eumenorrheic athletes and non-athletic controls. *Bone*, *45*(1), pp.104-109.
158. Scheid, J.L., De Souza, M.J., Hill, B.R., Leidy, H.J. and Williams, N.I., 2013. Decreased luteinizing hormone pulse frequency is associated with elevated 24-hour ghrelin after calorie restriction and exercise in premenopausal women. *American Journal of Physiology-Endocrinology and Metabolism*, *304*(1), pp.E109-E116.

159. Schneider, L.F. and Warren, M.P., 2006. Functional hypothalamic amenorrhea is associated with elevated ghrelin and disordered eating. *Fertility and sterility*, *86*(6), pp.1744-1749.
160. Schwertz, D.W. and Penckofer, S., 2001. Sex differences and the effects of sex hormones on hemostasis and vascular reactivity. *Heart & lung*, *30*(6), pp.401-428.
161. Skorupskaite, K., George, J.T. and Anderson, R.A., 2014. The kisspeptin-GnRH pathway in human reproductive health and disease. *Human reproduction update*, *20*(4), pp.485-500.
162. Scofield, K.L. and Hecht, S., 2012. Bone health in endurance athletes: runners, cyclists, and swimmers. *Current sports medicine reports*, *11*(6), pp.328-334.
163. Segrest, J.P., Li, L., Anantharamaiah, G.M., Harvey, S.C., Liadaki, K.N. and Zannis, V., 2000. Structure and function of apolipoprotein AI and high-density lipoprotein. *Current opinion in lipidology*, *11*(2), pp.105-115.
164. Shangold, M., Rebar, R.W., Wentz, A.C. and Schiff, I., 1990. Evaluation and management of menstrual dysfunction in athletes. *Jama*, *263*(12), pp.1665-1669.
165. Shufelt, C.L., Torbati, T. and Dutra, E., 2017, May. Hypothalamic amenorrhea and the long-term health consequences. In Seminars in reproductive medicine (Vol. 35, No. 03, pp. 256-262). Thieme Medical Publishers.
166. Silva, M.R. and Paiva, T., 2015. Low energy availability and low body fat of female gymnasts before an international competition. *European Journal of Sport Science*, *15*(7), pp.591-599.
167. Slater, J., McLay-Cooke, R., Brown, R. and Black, K., 2016. Female recreational exercisers at risk for low energy availability. *International journal of sport nutrition and exercise metabolism*, *26*(5), pp.421-427.
168. Smith, J.T., Acohido, B.V., Clifton, D.K. and Steiner, R.A., 2006. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *Journal of neuroendocrinology*, *18*(4), pp.298-303.
169. Snow-Harter, C.M., 1994. Bone health and prevention of osteoporosis in active and athletic women. *Clinics in sports medicine*, *13*(2), pp.389-404.
170. Soleimany G, Dadgostar H, Lotfian S, Moradi-Lakeh M, Dadgostar E, Movaseghi S (2012) Bone mineral changes and cardiovascular effects among female athletes with chronic menstrual dysfunction. Asian J Sports Med 3:53–58

171. Solomon, C.G., Hu, F.B., Dunaif, A., Rich-Edwards, J.E., Stampfer, M.J., Willett, W.C., Speizer, F.E. and Manson, J.E., 2002. Menstrual cycle irregularity and risk for future cardiovascular disease. *The Journal of Clinical Endocrinology & Metabolism*, *87*(5), pp.2013-2017.

172. Sonntag, B. and Ludwig, M., 2012. An integrated view on the luteal phase: diagnosis and treatment in subfertility. *Clinical endocrinology*, *77*(4), pp.500-507.

173. St-Pierre, D.H., Karelis, A.D., Cianflone, K., Conus, F., Mignault, D., Rabasa-Lhoret, R., St-Onge, M., Tremblay-Lebeau, A. and Poehlman, E.T., 2004. Relationship between ghrelin and energy expenditure in healthy young women. *The Journal of Clinical Endocrinology & Metabolism*, *89*(12), pp.5993-5997.

174. Spicer, L.J. and Francisco, C.C., 1997. The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. *Endocrinology*, *138*(8), pp.3374-3379.

175. Spieker, L.E., Sudano, I., Hürlimann, D., Lerch, P.G., Lang, M.G., Binggeli, C., Corti, R., Ruschitzka, F., Lüscher, T.F. and Noll, G., 2002. High-density lipoprotein restores endothelial function in hypercholesterolemic men. *Circulation*, *105*(12), pp.1399-1402.

176. Sundgot-Borgen, J., Torstveit, M.K. and Trainingcenter, O., 2003. The female athlete triad-the role of nutrition. *Schweizerische Zeitschrift fur Sportmedizin und Sporttraumatologie*, *51*(1), pp.47-52.

177. Tam, N., Santos-Concejero, J., Tucker, R., Lamberts, R.P. and Micklesfield, L.K., 2018. Bone health in elite Kenyan runners. *Journal of sports sciences*, *36*(4), pp.456-461.

178. Thompson, D.L., Snead, D.B., Seip, R.L., Weltman, J.Y., Rogol, A.D. and Weltman, A., 1997. Serum lipid levels and steroidal hormones in women runners with irregular menses. *Canadian journal of applied physiology*, *22*(1), pp.66-77.

179. Thong, F.S., McLean, C. and Graham, T.E., 2000. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. *Journal of Applied Physiology*, *88*(6), pp.2037-2044.

180. Tomazo-Ravnik, T. and Jakopič, V., 2006. Changes in total body water and body fat in young women in the course of menstrual cycle. *International Journal of Anthropology*, *21*(1), pp.55-60.

181. Tornberg, Å.B., Melin, A., Koivula, F.M., Johansson, A., Skouby, S., Faber, J. and Sjödin, A., 2017. Reduced neuromuscular performance in amenorrheic elite endurance athletes. *Medicine & Science in Sports & Exercise*, *49*(12), pp.2478-2485.
182. Torstveit, M.K. and Sundgot-Borgen, J., 2005. Participation in leanness sports but not training volume is associated with menstrual dysfunction: a national survey of 1276 elite athletes and controls. *British journal of sports medicine*, *39*(3), pp.141-147.
183. Ulloa, N., Arteaga, E., Bustos, P., Dur, D., Schulze, K., Castro, G., Jauhiainen, M., Fruchart, J.C. and Calvo, C., 2002. Sequential estrogen-progestin replacement therapy in healthy postmenopausal women: effects on cholesterol efflux capacity and key proteins regulating high-density lipoprotein levels. *Metabolism-Clinical and Experimental*, *51*(11), pp.1410-1417.
184. VanHeest, J.L., Rodgers, C.D., Mahoney, C.E. and De Souza, M.J., 2014. Ovarian suppression impairs sport performance in junior elite female swimmers. *Medicine and science in sports and exercise*, *46*(1), pp.156-166.
185. Vulliémoz, N.R., Xiao, E., Xia-Zhang, L., Germond, M., Rivier, J. and Ferin, M., 2004. Decrease in luteinizing hormone pulse frequency during a five-hour peripheral ghrelin infusion in the ovariectomized rhesus monkey. *The Journal of Clinical Endocrinology & Metabolism*, *89*(11), pp.5718-5723.
186. Wade, G.N. and Jones, J.E., 2004. Neuroendocrinology of nutritional infertility. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *287*(6), pp.R1277-R1296.
187. Wade, G.N. and Schneider, J.E., 1992. Metabolic fuels and reproduction in female mammals. *Neuroscience & Biobehavioral Reviews*, *16*(2), pp.235-272.
188. West, R.V., 1998. The female athlete. *Sports Medicine*, *26*(2), pp.63-71.
189. Williams, N.I., Caston-Balderrama, A.L., Helmreich, D.L., Parfitt, D.B., Nosbisch, C. and Cameron, J.L., 2001. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: abrupt transition to exercise-induced amenorrhea. *Endocrinology*, *142*(6), pp.2381-2389.
190. Williams, N.I., Helmreich, D.L., Parfitt, D.B., Caston-Balderrama, A. and Cameron, J.L., 2001. Evidence for a causal role of low energy availability in the induction of

menstrual cycle disturbances during strenuous exercise training. *The Journal of Clinical Endocrinology & Metabolism*, *86*(11), pp.5184-5193.

191. Williams, P.T., 1996. High-density lipoprotein cholesterol and other risk factors for coronary heart disease in female runners. *New England Journal of Medicine*, *334*(20), pp.1298-1304.
192. Wheatley, S., Khan, S., Székely, A.D., Naughton, D.P. and Petróczi, A., 2012. Expanding the female athlete triad concept to address a public health issue. *Performance Enhancement & Health*, *1*(1), pp.10-27.
193. Wimpfheimer, C., Saville, E., Voirol, M.J., Danforth, E. and Burger, A.G., 1979. Starvation-induced decreased sensitivity of resting metabolic rate to triiodothyronine. *Science*, *205*(4412), pp.1272-1273.
194. Wren, A.M., Small, C.J., Ward, H.L., Murphy, K.G., Dakin, C.L., Taheri, S., Kennedy, A.R., Roberts, G.H., Morgan, D.G.A., Ghatei, M.A. and Bloom, S.R., 2000. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology*, *141*(11), pp.4325-4328.
195. Yenilmez, M.I., 2020. CRITICAL THOUGHTS AND INSIGHTS ON THE FEMALE ATHLETE TRIAD: PRECEDENTS, EXISTING CHALLENGES AND PROSPECTS.
196. Yeo, S.H. and Colledge, W.H., 2018. The Role of Kiss1 Neurons As integrators of endocrine, Metabolic, and environmental Factors in the Hypothalamic–Pituitary–Gonadal Axis. *Frontiers in endocrinology*, *9*, p.188.
197. Young, N.I.C.H.O.L.A.S., Formica, C.A.R.M.E.L.O., Szmukler, G.E.O.R.G.E. and Seeman, E., 1994. Bone density at weight-bearing and nonweight-bearing sites in ballet dancers: the effects of exercise, hypogonadism, and body weight. *The Journal of Clinical Endocrinology & Metabolism*, *78*(2), pp.449-454.
198. Zanker, C.L., Osborne, C., Cooke, C.B., Oldroyd, B. and Truscott, J.G., 2004. Bone density, body composition and menstrual history of sedentary female former gymnasts, aged 20–32 years. *Osteoporosis International*, *15*(2), pp.145-154.