

Thesis - StrongHER Project: Exploring the Impact of Nutrition on Bone Health in Female Athletes

Principal Investigator: Katriona Ross
Co-Investigator(s):
Dr Fergus Guppy (Associate Professor)
Dr Hannah Lithgow (Assistant Professor)
Dr David King (Assistant Professor)

2024-12-11

Contents

1	Introduction	2
1.1	What is already known/reported on this topic?	2
1.2	What could this study add?	2
1.3	Potential for this study to affect practice, research or policy	2
2	Background & Rationale	2
2.1	Overview of Bone Health in Female Athletes	2
2.2	Importance of Vitamin D in Bone Health	3
2.3	Prevalence of Vitamin D Deficiency in Female Athletes	4
2.4	Effects of Vitamin D on Bone Health in Female Athletes	4
2.5	Bone Turnover Markers and Stress Fracture Risk	4
2.6	Biological Differences	5
2.7	Energy Availability and Relative Energy Deficiency in Sport (RED-S)	6
2.8	Prevalence of RED-S in Female Athletes	7
2.9	Leptin and It's Role as a Biomarker of Energy Availability	7
2.10	Leptin's Role in Bone Metabolism	8
2.11	Sex Difference sin Leptin Responses and Implications for Female Athletes	8
3	Study Aims	9
3.1	Study Objectives	9
3.2	Expected Outcomes	9
4	Materials and Methods	10
4.1	Study Design	10
5	Participants	10
6	Ethics	11
	References	11

1 Introduction

1.1 What is already known/reported on this topic?

1. It is known that low energy availability (LEA) because of insufficient calorie intake or over training can lead to amenorrhea and bone health conditions such as low bone mineral density (BMD) or osteoporosis long term.
2. It is known that inadequate intake of essential nutrients such as calcium, vitamin D will significantly affect BMD.
3. It is known that both LEA and poor micronutrient intakes are common in female athletes who are at further increased risk if they are training indoors or live in norther latitudes.

1.2 What could this study add?

1. Longitudinal data tracking changes in bone health, energy availability and nutrient intakes over time.
2. More high-quality research in female athletes, an understudied population.
3. Research across different sporting populations i.e. indoor vs outdoor, impact vs non-impact.
4. Investigate the efficacy and use of BIA for early detection of LEA compared to currently used diagnostic criteria.
5. Could help identify potential future interventions for nutrition in female athletes for improved bone health and energy availability.

1.3 Potential for this study to affect practice, research or policy

The proposed study holds significant implications for advancing our understanding of energy availability (EA), micronutrient status and bone health in female athletes. By addressing these critical gaps in knowledge, the research aims to optimise both health and performance outcomes. The findings will not only contribute to academic knowledge but also provide practical insights for coaches, nutritionists, and practitioners working with female athletes.

2 Background & Rationale

Research shows a substantial under-representation of women with only 6-12% of all studies utilising female-only participants (Cowley et al. 2021; Hutchins et al. 2021). Furthermore, only 10% of the studies used elite or highly trained athletes (Smith et al. 2022). This underrepresentation of female athletes in sport science and sport medicine (SSSM) research may have significant implications on our understanding of the unique physiological factors impacting female athlete health and performance. Additionally, the methodological quality of studies conducted on female athletes has been compromised due to historical neglect and a lack of understanding of reproductive endocrinology. Traditionally, sport and exercise science data have primarily originated from studies involving male participants, resulting in a dearth of high-quality data applicable to female athletes (Elliott-Sale et al. 2021). Applying evidence developed based on male athlete research to female cohorts may be erroneous and with significant challenges (Emmonds, Heyward, and Jones 2019)

Therefore, one of the aims of this study is to produce high-quality female-specific data through a more comprehensive and tailored approach to research, ultimately contributing to a more robust understanding of the unique physiological considerations in female athletes.

2.1 Overview of Bone Health in Female Athletes

Athletes, particularly female athletes are at increased risk of developing bone injuries and bone-related conditions, such as stress fractures, osteoporosis and osteopenia due to imbalance between training and recovery

(Goolsby and Boniquit 2017). Bone health is multifactorial, adequate nutrition, sufficient mechanical stimulus, hormonal, genetic and environmental factors all playing a role in achieving and maintaining optimal bone health in athletes. The primary concern for athletes and support staff being the avoidance of injury as this will directly influence performance. In addition to affecting athletic performance, optimal bone health is essential for long-term well-being of female athletes as poor bone health can lead to more chronic conditions later in life.

At present, osteoporosis affects 1 in 3 women compared to 1 in 5 men. Additionally, the number of hip fractures in the UK due to osteoporosis has risen from 70,000 in 2006 to 100,000 in 2020, an increase of 44% (Stevenson 2023). Consequently, there is both a significant economic burden and a clear impact on the quality of life of those affected. The heightened vulnerability of female athletes to bone injuries and related conditions underscores the crucial role of nutrition in fostering optimal bone health.

Stress fractures and bone stress injuries, resulting from overuse and increased mechanical loading are common in many sports (McBryde 1985), with these injuries being complex and not completely understood, current evidence suggests that dietary restraint, suboptimal vitamin D status ($<30\text{ng/ml}$) and menstrual dysfunction are risk factors for sustaining bone stress injuries (Barrack et al. 2014; Bishop et al. 2021; Neal et al. 2015). Vitamin D plays a crucial role in calcium absorption and bone metabolism making it a key nutrient for bone health.

****Explain here some context of why BMD is a useful marker of skeletal health would be useful**

The interplay between diet and exercise training is also critical in better understanding the long-term bone health of retired athletes, with the avoidance of conditions such as osteopenia and osteoporosis crucial in maintaining a healthy lifespan in this population. This involves maximising peak bone mineral density (BMD), which relates to the strength of the internal bone tissue, in relation to microarchitecture and geometry and managing the rate of bone loss with increasing age (Bailey and Brooke-Wavell 2008). As ninety percent of peak bone mass is accrued by age 20 and then, after a period of stability BMD will begin to decline as women reach their late 30's. Men not only exhibit higher total BMD but are also less susceptible to age related bone loss than women (Santos, Elliott-Sale, and Sale 2017). Although very little research has been completed on athletes' long term bone health post career, studies suggest that athletes maintain higher bone density than controls (Bass et al. 1998; Kirchner, Lewis, and O'Connor 1996). A detraining study in former female gymnasts found that at follow up, BMD remained 16% greater than controls at the proximal femur and 5% in the total body but not different at the lumbar spine (Kudlac et al. 2004). The rate of BMD change per year was also only greater between groups at the lumbar spine (gymnasts: -0.8% vs controls: 0.2%), highlighting the importance of maximising premenopausal BMD to reduce the risk of osteoporosis and fractures later in life.

While various dietary and nutritional practices have been proposed to improve bone health in athletes, including vitamin D and calcium supplementation, the female-specific effects of micronutrients on bone health in female athletes remain unclear Smith et al. (2022). An audit of studies supporting the use of calcium, iron and vitamin D found that only 32% of studies defined menstrual status, with none implementing best practice protocols.

2.2 Importance of Vitamin D in Bone Health

Vitamin D is synthesised in the skin when exposed to sunlight. Ultraviolet-B (UVB) radiation converts 7-dehydrocholesterol into vitamin D3 (Cholecalciferol). This newly synthesised vitamin D is then transported to the liver by Vitamin D binding protein (VDBP). In the liver, it is converted to $25(\text{OH})\text{D}$, which is the main storage form of vitamin D. In the kidney, further activation occurs, resulting in $1,25(\text{OH})_2\text{D}$, the active form. This activation is driven by parathyroid hormone (PTH) when blood calcium and/or phosphate concentrations fall below normal ranges.

There are two main types of vitamin D that are available in the diet: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2). Cholecalciferol is synthesised in the skin when exposed to sunlight and is also found in animal-based food sources such as fatty fish and fish liver oils. Ergocalciferol, on the other hand, is derived from plants and is found in foods such as mushrooms exposed to ultraviolet light and fortified

plant-based milk alternatives. Both cholecalciferol and ergocalciferol, can contribute to the body’s vitamin D levels when consumed through the diet. These are well absorbed (bioavailability of 50%) especially when consumed along with dietary lipids (E. Larson-Meyer 2015)

Vitamin D plays a crucial role in bone health by facilitating calcium absorption in the gut and helping maintain adequate serum calcium and phosphate concentrations for normal bone mineralisation. Vitamin D also plays a role in the prevention of rickets in children, osteomalacia in adults and in conjunction with calcium helps prevent the onset of osteoporosis in older adults (Holick 2007).

2.3 Prevalence of Vitamin D Deficiency in Female Athletes

The prevalence of vitamin D deficiency among athletes varies based on factors such as season, training location, sport (D. E. Larson-Meyer and Willis 2010), and skin colour (Hamilton 2010). Several studies have highlighted the common issue of vitamin D deficiency in athletes. Seasonal variation of serum vitamin D (25 (OH) D) was measured in elite male athletes and over 65% of participants had insufficient ($<50\text{nmol/L}$) levels during the winter (Morton et al. 2012). Similarly, UK university athletes, especially those who spend significant time indoors, are at risk of vitamin D deficiency, emphasising the need for further research to understand its implications on their health and performance (Wilson-Barnes et al. 2020). Adolescent female soccer players were also found to have insufficient levels of vitamin D with a mean value of all participants reported to be $50.5 \pm 12.8 \text{ nmol/L}$ (Brännström et al. 2016). Jakše et al. (2021) reported that only 32.3% of athletes had sufficient levels of 25(OH)D ($>75 \text{ nmol/L}$), with gymnasts exhibiting lower sufficiency compared to swimmers (24% in gymnasts vs 43% in swimmers). These findings highlight the importance of interventions to address vitamin D deficiency among athletes.

2.4 Effects of Vitamin D on Bone Health in Female Athletes

Optimal vitamin D levels are essential for individuals, with particular significance for female athletes. Beyond its established role in calcium absorption and skeletal health, recent research highlights the broader implications of sufficient vitamin D status for athletes. Maintaining adequate vitamin D levels has been linked to improved muscle function, reduced inflammation, and enhanced immune response, crucial factors in achieving peak athletic performance (Berry et al. 2011; G. L. Close et al. 2012; Owens, Allison, and Close 2018).

The evidence demonstrating the relationship between vitamin D and lower body muscle strength and power is mixed. Some papers found no effect of vitamin D on muscle strength or power [Bezuglov et al. (2023); G. L. Close et al. (2012); Jastrzębska, Kaczmarczyk, and Jastrzębski (2016); Todd et al. (2017)] whereas others found opposite effects (Alimoradi et al. (2019); Graeme L. Close et al. (2013); Jung et al. (2018); Rockwell et al. (2020); Wyon et al. (2016); Wyon et al. (2019)). To the authors knowledge, there were only seven papers who used female participants in varying percentages (40% to 69%) with one not even disclosing the sex of the participants involved. However, none used exclusively female participants. This is a significant gap in the literature and one this study aims to fill.

For female athletes, the importance of optimising vitamin D levels extends to hormonal regulation, including potential effects on oestrogen levels (Chu et al. 2021). This connection may influence menstrual regularity and bone health, addressing concerns, related to relative energy deficiency in sport (RED-s). Furthermore, there is evidence that vitamin D may affect fertility. There are vitamin D receptors (VDRs) present in the ovaries and uterus, suggesting a direct role in the reproductive process (Lerchbaum and Obermayer-Pietsch 2012). In this context, elevating vitamin D levels is crucial for female athletes as part of a comprehensive strategy to support musculoskeletal performance, hormonal balance, and overall well-being, contributing to sustained athletic excellence and long-term health.

2.5 Bone Turnover Markers and Stress Fracture Risk

Vitamin D is primarily known for its role in supporting calcium absorption, and low mechanistic action of vitamin D explains why low circulating levels can reduce bone strength and increase stress fracture risk. Low levels of 25(OH)D normally accompany low calcium levels, resulting in PTH secretion to meet the demand.

This results in increased bone turnover, reduced bone mass and potentially increased risk of stress fracture, especially in athletes (Yagüe et al. 2020). Most of the research in this area has been undertaken in the military. Stress fracture incidence in recruits has been estimated between 0.2% and 5.2% in men and 1.6% and 30% in women (Ribbans et al. 2021). Vitamin D deficiency has been associated with stress fractures in female athletes (Jakobsen et al. 2021; Knechtle et al. 2021; Millward et al. 2020; Moreira and Bilezikian 2017). Moreover, supplementation with vitamin D, often in combination with calcium is shown to reduce the risk of stress fracture in female athletes (Lappe et al. 2008; Millward et al. 2020; Tenforde et al. 2010). This highlights the importance of vitamin D in maintaining bone strength and reducing injury risk in all athletes, especially female athletes.

2.6 Biological Differences

There are clear biological differences between males and females that extend beyond reproductive functions. These differences include variations in hormone levels, body composition, and musculoskeletal structure. These biological differences play a crucial role in influencing the nutritional needs and metabolic processes of female athletes (Chen et al. 2022). Understanding these differences is essential for developing tailored dietary and training strategies that optimise performance and promote long-term health in female athletes.

Female athletes typically exhibit elevated body fat levels compared to their male counterparts, whereas males typically possess greater lean body mass and muscle mass. These variations in body composition can influence bone health in female athletes (Sanborn and Jankowski 1994). For example, differences in fat mass and lean body mass may impact how bones are mechanically loaded during physical activity, which can then affect bone remodelling processes (Jürimäe, Gruodyte-Racine, and Baxter-Jones 2018). Furthermore, variations in body fat percentage may influence hormonal regulation, particularly oestrogen levels, which are pivotal in bone metabolism [Mair, Gaw, and MacLean (2020); Narla and Ott (2018)]. Thus, a comprehensive understanding of these sex-specific differences is crucial for developing targeted interventions to optimise bone health and energy balance among female athletes, ultimately improving their overall performance and wellbeing.

Research consistently demonstrates that males typically have a higher resting metabolic rate (RMR) and total energy expenditure compared to females, again this is largely attributed to differences in body mass and body composition (Arciero, Goran, and Poehlman 1993; Jagim et al. 2023). The differences in RMR and exercise energy expenditure between males and females can create significant differences in total energy demands and the necessary energy intakes. These differences can lead to instances of inadequate energy availability (EA), which can negatively influence physiological functions, including bone metabolism (Sale and Elliott-Sale 2019). By measuring both RMR and assessing energy expenditure, this study aims to elucidate how these variances may influence EA and, consequently, bone health in female athletes.

EA is defined as energy intake (EI) less exercise energy expenditure (EEE) relative to fat free mass (FFM) ($EA = (EI - EEE) / FFM$). EI is assessed by measuring total caloric intake from all food and drink consumed. Common methods of measurement include 24-hour dietary recall, food frequency questionnaires and food diaries. Among these methods, weighted food diaries, if completed correctly allow for accurate estimation of energy intake. They provide the researcher or practitioner with detailed quantitative information of macro and micronutrient intakes. However, this methodology does have significant limitations. The process is incredibly time consuming and the burden of weighing and recording every single food item can lead to poor compliance especially during busy training periods. As with most methods of dietary analysis the potential for underreporting food intake or altering their eating habits is a possibility. Despite the time-consuming nature for the participants, weighted food records show high rates of accuracy and reliability (Thompson and Subar 2017) and with sufficient education and support throughout the process it is a method that can be successfully implemented in an athletic population.

EEE represents the amount of energy used during physical activity. This is estimated through accelerometry devices, heart rate (HR) monitoring or indirect calorimetry during exercise. The final component, FFM can be calculated using whole body dual X-ray absorptiometry (DXA) scanners, bioelectrical impedance analysis (BIA) or skinfold callipers. As a critical variable in the EA equation, FFM is measured most accurately using the gold standard method, DXA scan (Nana et al. 2015).

Although not directly included in the EA equation, RMR is a key component in calculating EA, representing the energy needed to maintain vital physiological functions at rest and the main contributor to total daily energy expenditure (TDEE). Accurate measurement of RMR helps improve TDEE estimates, leading to more precise calculations of EA.

Understanding the interplay between EA and bone health will help both researchers and practitioners to identify risk factors and prevention strategies for low energy availability (LEA) and support skeletal health in female athletes, therefore, enhancing performance and minimising the risk of bone-related injuries and conditions.

Studying the hormonal differences in male and female athletes, both throughout the menstrual cycle and in everyday life, is crucial for researchers to develop a thorough understanding of female athletes. Females experience cyclical fluctuations in oestrogen and progesterone levels throughout the menstrual cycle, which can influence bone density and strength (Rugvedh, Gundreddy, and Wandile 2023). Additionally, differences in baseline hormonal profiles between males and females, such as higher levels of testosterone in males and fluctuations in insulin and leptin levels, contribute to variations in body composition, metabolic rate, and energy balance (Lundsgaard and Kiens 2014). These hormonal differences have direct implications for bone mineral density in female athletes (Gruodytė et al. 2010). Therefore, any study using a female athlete population should aim to measure these variables.

Considering the evident biological differences between males and females, with distinct implications for nutrition, it is crucial to acknowledge these sex-based differences in the context of female athlete research. There is a clear need for female athlete-specific research to ensure there is adequate support for both their health and performance.

2.7 Energy Availability and Relative Energy Deficiency in Sport (RED-S)

Conducting a study exploring the influence of nutrition on female athlete bone health stems from the critical importance of increasing our understanding of these interconnected factors in optimising athletic performance and preventing long-term bone health issues (e.g. risk of osteoporosis and osteopenia). Female athletes often face unique challenges related to bone health and nutrition, specifically related to EA. Intense physical training and inadequate nutrition may contribute to the development of RED-S. The term RED-S was introduced in 2014 as a replacement for the previous term Female Athlete Triad, as it was found to affect both males and females. Although this thesis will include literature based on the previous model, for the purposes of this thesis it will be referred to as RED-S. Since then, over 170 research publications have been published to advance the field, leading to updated conceptual models and definitions. The current definition of RED-S is:

“A syndrome of impaired physiological and/or psychological functioning experienced by both female and male athletes. It is caused by exposure to problematic (prolonged and/or severe) LEA. The detrimental outcomes include, but are not limited to, decreases in energy metabolism, reproductive function, musculoskeletal health, immunity, glycogen synthesis, and cardiovascular and haematological health. These outcomes can individually and synergistically lead to impaired well-being, increased injury risk, and decreased sports performance.”

Understanding the dynamic interplay between bone health, EA, and micronutrient status over time is crucial for developing targeted interventions and preventive strategies to safeguard the well-being and performance of female athletes and informing best practice in performance sport.

The risks of sustained periods of LEA are clear, with no positive outcomes. In terms of bone health, an energy deficit can disrupt hormones, decrease osteoblast activity, and increase bone resorption. This imbalance in bone turnover can then reduce the capacity to lay down newly formed bone and impair the ability to repair micro damage (Barrack et al. 2014). Areta et al., (2021) highlighted that LEA results in greater suppression of markers of bone formation and increased markers of bone resorption and this effect has been documented in females but not in males. These responses are thought to precede the clinical complications, reproductive function impairment, stress fractures, loss of muscle mass and impaired physical capacity. Early findings

suggest that men may be less sensitive to reductions in EA than females however, most studies reviewed were short term (< 5 days)(Ihle and Loucks 2004; Loucks and Heath 1994; Papageorgiou et al. 2017, 2018).

Current understanding is that any drop in energy availability below a threshold of 30kcal/kg/FFM per day in females is sub-optimal and going to result in an acute disruption of normal hormonal, endocrine, physiological and metabolic function (Areta, Taylor, and Koehler 2021).

Need for Reliable Tools to Identify Athletes at Risk of LEA and RED-S

Detecting those athletes at risk of LEA as early as possible is key to avoiding negative health consequences in the future. Therefore, necessary tools for screening are required. Questionnaires present a practical, non-invasive and time effective method of screening athletes. Various questionnaires currently exist such as the Eating Disorder Examination Questionnaire (EDE-Q), Brief Eating Disorder in Athletes Questionnaire (BEDA-Q), RED-S Specific Screening Tool (RST) and Three Factor Eating Questionnaire (TFEQ). However, many of these tools such as the BEDA-Q were not designed specifically for female athletes and have limitations. For example, the EDE-Q cut-off scores may vary between sexes and its reliance on behavioural indicators limits the ability to detect the full scope of LEA and RED-S (Sim and Burns 2021). The Low Energy Availability in Females Questionnaire (LEAF-Q) is a validated questionnaire that focusses specifically on female athletes' health and performance. The LEAF-Q has been validated in studies with elite and highly trained populations in sports such as triathlon (Witkoś, Błażejowski, and Gierach 2023), dance, endurance, (Melin et al. 2014) and combat sports (Rogers et al. 2021). It has a 90% specificity and 78% sensitivity in successfully identifying RED-S. Developed by Melin et al., 2014, the LEAF-Q assesses menstrual function, gastrointestinal health and injuries, factors all strongly linked to the development of LEA and RED-S. A criticism of the LEAF-Q is that it does not consider EEE. While this tool should not be used to diagnose LEA or RED-S, it may provide additional context for researchers and practitioners in order to conduct further investigations i.e. clinical testing. Prior to 2015 LEA was predominantly identified by using a combination of weighted food diaries and training logs in conjunction with heart rate or accelerometry data. Since then, it has been established that LEA can be easily and accurately identified utilising surrogate markers such as RMR ratio, LEAF-Q and biomarker analysis [Logue et al. (2020); Heikura et al. (2018)].

2.8 Prevalence of RED-S in Female Athletes

Symptoms of RED-S and LEA were more prevalent amongst professional female athletes compared to sedentary, recreationally active controls (Skarakis et al. 2021).

| ** Could add in BIA section and Phase Angle here too

2.9 Leptin and It's Role as a Biomarker of Energy Availability

Leptin, a key hormone produced by adipose tissue, regulates energy balance, metabolism, immune function and other systems by influencing hypothalamic control and exerting effects on peripheral tissues (Park and Ahima 2015). Leptin is correlated with body fat mass and nutritional status, indicating energy availability or deficiency in the body.

Evidence suggests that leptin fluctuates in line with menstrual cycle phases, particularly in naturally menstruating individuals which may reflect underlying changes in energy balance and metabolism. Lofberg et al.(2024) compared the effects of endogenous and exogenous female sex hormones on resting energy expenditure and metabolic hormones in two hormonally distinct groups. Leptin levels were significantly lower in the luteal phase, compared to the follicular phase, aligning with simultaneous reductions in T3. This was despite an overall increase in REE.

Athletes with menstrual disorders have decreased T3 and REE which may indicate LEA (Moto et al. 2022). Similarly, many studies measuring leptin in states of LEA show a decrease with values below 30kcal/kg/FFM independent of whether it is achieved through exercise or food restriction (Areta, Taylor, and Koehler 2021). Insulin is a downstream marker of leptin and suggested to play a role in sensing energy balance within the body and be a determinant of BMD in females (Maya and Misra 2022).

Bone health can be significantly impacted by the reduction in leptin caused by LEA. Reduced leptin levels, results in a two-fold effect, in that, bone resorption increases, and formation is decreased, which leads to

greater losses in bone density and higher risk of injury and fracture.

2.10 Leptin's Role in Bone Metabolism

A review by Upadhyay et al. (2015) suggests leptin may exert both direct and indirect effects on bone. Directly, leptin receptors on chondrocytes and osteoblasts suggest that leptin may influence bone formation by binding to receptors and activating a signalling pathway to promote osteoblast differentiation and activity.

Indirectly, leptin influences bone metabolism via the central nervous system (CNS). It stimulates pro-opiomelanocortin (POMC) neurons while suppressing agouti-related peptide (AgRP) and neuropeptide Y (NPY) neurons in the hypothalamus, which work concurrently to suppress appetite and affect energy expenditure (Park and Ahima 2015). This allows leptin to control energy and hormone levels to maintain optimal bone health.

Leptin increases production of osteoblasts which increases the production of osteocalcin. Osteocalcin is a key regulator in bone metabolism and insulin sensitivity. Specifically, when leptin is increased, this stimulates the production of osteocalcin which triggers the release of insulin from the pancreas. This acts as a positive feedback loop for bone formation. Moreover, when released from adipose tissue, leptin increases serotonin production, leading to increased bone growth via enhanced osteoblast proliferation and reduced osteoclast activity.

Leptin also activates estrogen production via the hypothalamic-pituitary-gonadal axis. Leptin signals the hypothalamus to release gonadotropin releasing hormone (GnRH), which triggers the pituitary gland to secrete luteinising hormone (LH) and follicle stimulating hormone (FSH). The increase in these hormones results in the ovaries producing estrogen which in turn promotes the growth of osteoblasts while reducing cortisol levels to help mitigate the catabolic effects of cortisol on bone. All these factors make leptin an ideal biomarker to help identify athletes at risk of LEA and the associated effects on bone health. Murphy et al. (2021) suggested that decreases in leptin are greater in conditions of LEA compared to control. Each participant experienced three different conditions for five days, LEA and high protein, LEA and low protein or control. The decline in leptin in response to LEA over the five-day period was $54.3\% \pm 16.7\%$, $65.5\% \pm 4.4\%$ and $25.4\% \pm 11.4\%$ respectively. Although in a population of young, healthy males, this study highlights that LEA is associated with reductions in circulating leptin levels, which may have contributed to the shift from bone turnover to bone resorption although this has not been directly explored.

2.11 Sex Difference in Leptin Responses and Implications for Female Athletes

When it comes to bone health, the consequences of LEA appear to be sex specific with female athletes experiencing more profound effects. Male athletes may be less affected partly because of lower energy demand for reproductive function (Papageorgiou et al. 2017) and the bone-protective effects of testosterone (Souza et al. 2014; Thomas et al. 2001). In a study by Papageorgiou et al. (2017) they examined the effects of LEA, induced through reduced dietary intake and exercise in physically active males and females. In males, there was no significant differences in bone turnover markers such as Procollagen type 1 N propeptide (P1NP) ($p=0.12$) and C-terminal cross linked telopeptide of type 1 collagen (BCTX) ($p=0.46$) between restriction and control. Hormonal markers including insulin ($p=0.40$) and leptin ($p=0.07$) were also not significantly different in males in restricted compared to control. In females, they found significant changes in bone turnover markers, with P1NP significantly lower ($p=0.01$) and BCTX was significantly higher ($p=0.03$) in restriction compared to control conditions. The hormonal markers including insulin and leptin were also significantly reduced in females under the restricted condition ($p=0.01$). The findings suggest that in females in conditions of LEA, bone formation is decreased, and resorption is increased. Key hormonal markers were also heavily impacted. However, in males, LEA has a minimal impact on bone and hormonal markers, indicating their greater resilience to LEA. Supporting studies by Hickey et al. (1997) and Dubac et al. (1998) show significant bone metabolism impacts from LEA in females but not in males. This suggests that LEA's detrimental effects on bone health may be sex-specific, necessitating a tailored approach to managing athletes at risk of LEA-related complications. The impact of leptin in female athlete populations has not been extensively studied. Research on leptin replacement therapy in females with hypothalamic amenorrhea (HA) has shown promising results. Sienkiewicz et al. (2011) observed that Metreleptin, a leptin replacement,

significantly improved bone mineral density (BMD) and bone mineral content (BMC) at the lumbar spine, along with increased IGF-1 and reduced cortisol levels. Given these outcomes, more research into leptin's role in female athletes, especially those prone to LEA and associated bone conditions, is recommended.

3 Study Aims

There is limited understanding of the influence of nutrition on bone health in elite female athlete populations. The aim of this study is to investigate the physiological, physical, and nutritional characteristics of elite and highly trained female athletes based in Scotland, with a focus on bone health and nutrition-related factors to gain a better understanding of the female athlete. A wide range of factors will be explored to determine what, if any, future interventions may optimise bone health and overall performance among this population.

3.1 Study Objectives

1. To produce high-quality female-specific data through a more comprehensive and tailored approach to research, ultimately contributing to a more robust understanding of the unique physiological considerations in female athletes living in Scotland.
2. Evaluate the impact of micronutrient intake, with a specific focus on vitamin D, on bone health in female athletes. Through a comprehensive analysis of dietary patterns and serum micronutrient levels, the objective is to discern any significant associations between specific micronutrient profiles and markers of bone health, providing valuable insights into potential nutritional interventions for the prevention of bone injuries and conditions.
3. To assess the association between vitamin D intakes and reductions in negative menstrual cycle symptoms in female athletes
4. To explore the status of bone health in a population of female athletes using markers of bone formation/resorption and dual x-ray absorptiometry (DXA).
5. To examine the relationship between body composition and bone health status in female athletes using DXA.
6. Investigate the efficacy of bioelectrical impedance analysis (BIA) as a potential method of early detection and diagnosis of LEA.
7. To evaluate the impact of energy availability on bone health in female athletes by analysing data from weighted food diaries and energy expenditure assessments and exploring the association with BMD
8. To assess the prevalence of bone-related injuries and conditions among female athletes and explore potential risk factors using questionnaire data given at the baseline screening.

3.2 Expected Outcomes

1. There is a significant association between vitamin D intake and bone health in female athletes, with adequate levels of vitamin D correlating with improved bone density and reduced markers of bone resorption.
2. Specific micronutrient profiles, particularly high intakes of calcium and vitamin D are significantly associated with positive markers of bone health, such as increased bone formation and decreased bone resorption, in female athletes
3. There will be a significant negative correlation between energy availability and markers of bone resorption in female athletes.
4. BIA is an effective and reliable method for early detection and diagnosis of LEA in female athletes, as evidenced by significant correlations between measurements of phase angle, body composition and clinical markers of LEA.

5. Body composition, specifically, low levels of fat mass in female athletes as measured by DXA, will correlate with reduced bone formation markers, increased bone resorption and decreased BMD.
6. Bone related injuries will be higher in female athlete populations with specific risk factors, including low fat mass, decreased leptin and LEA below 30kcal/kg/FFM.

4 Materials and Methods

4.1 Study Design

This study will be an observational repeated measures design with data collected from participants across a rolling 12-month period, as shown in Figure 1. Due to the seasonal nature of vitamin D synthesis participant visits will be separated by three months to ensure data collection occurs in all four UK seasons (Macdonald et al. 2011)

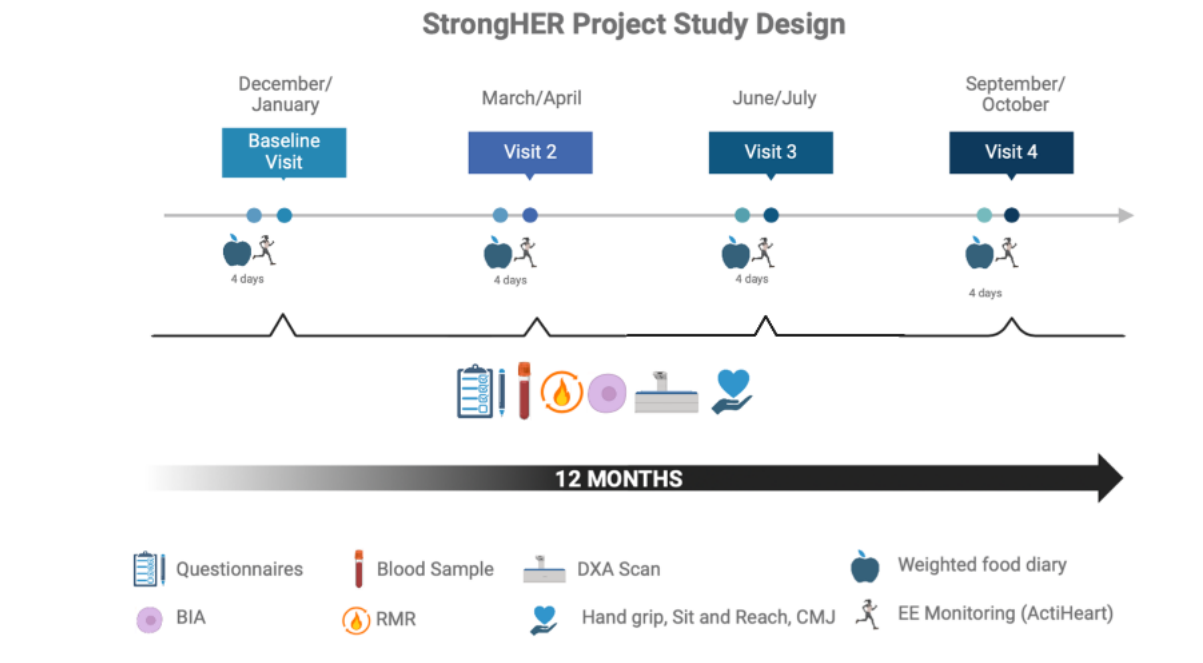


Figure 1: StrongHER Study Design

5 Participants

Participants will be female, aged between >18 and <40 years. Participants will be defined as healthy as determined by a health screening questionnaire. No use of medication that could affect bone metabolism such as glucocorticoids (e.g., prednisone, cortisone). Participants exceeding the weight limit for the DXA scanner will be excluded (>204kg/450lbs). Further exclusion criteria include any females who are currently pregnant or participants who have breastfed within the last 12 months. Participants' training status will be classified using the criteria outlined by McKay et al. (2021) with supporting classification provided. Participants will be Tier 3 (National level/highly trained) and above. Participants' menstrual status will be reported in line with the tiering system proposed by Elliott-Sale et al. (2021). All participants will receive a participant information sheet via email and have a minimum of 24 hours to read through the information

sheet. Participants will then be given the opportunity to ask questions prior to providing written informed consent.

6 Ethics

Ethical approval was approved by the Energy, Geoscience, Infrastructure and Society Ethics Committee of Heriot Watt University (Dec 2024).

References

- Alimoradi, Karamollah, Bahareh Nikooyeh, Ali Asghar Ravasi, Maliheh Zahedirad, Nastaran Shariatzadeh, Ali Kalayi, and Tirang Reza Neyestani. 2019. "Efficacy of Vitamin D Supplementation in Physical Performance of Iranian Elite Athletes." *International Journal of Preventive Medicine* 10 (June): 100. https://doi.org/10.4103/ijpvm.IJPVM_227_18.
- Arciero, P. J., M. I. Goran, and E. T. Poehlman. 1993. "Resting Metabolic Rate Is Lower in Women Than in Men." *Journal of Applied Physiology (Bethesda, Md.: 1985)* 75 (6): 2514–20. <https://doi.org/10.1152/jappl.1993.75.6.2514>.
- Areta, José L., Harry L. Taylor, and Karsten Koehler. 2021. "Low Energy Availability: History, Definition and Evidence of Its Endocrine, Metabolic and Physiological Effects in Prospective Studies in Females and Males." *European Journal of Applied Physiology* 121 (1): 1–21. <https://doi.org/10.1007/s00421-020-04516-0>.
- Bailey, C. A., and K. Brooke-Wavell. 2008. "Exercise for Optimising Peak Bone Mass in Women: Post-graduate Symposium." *Proceedings of the Nutrition Society* 67 (1): 9–18. <https://doi.org/10.1017/S0029665108005971>.
- Barrack, Michelle T., Jenna C. Gibbs, Mary Jane De Souza, Nancy I. Williams, Jeanne F. Nichols, Mitchell J. Rauh, and Aurelia Nattiv. 2014. "Higher Incidence of Bone Stress Injuries With Increasing Female Athlete Triad-Related Risk Factors: A Prospective Multisite Study of Exercising Girls and Women." *The American Journal of Sports Medicine* 42 (4): 949–58. <https://doi.org/10.1177/0363546513520295>.
- Bass, S., G. Pearce, M. Bradney, E. Hendrich, P. D. Delmas, A. Harding, and E. Seeman. 1998. "Exercise Before Puberty May Confer Residual Benefits in Bone Density in Adulthood: Studies in Active Prepubertal and Retired Female Gymnasts." *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research* 13 (3): 500–507. <https://doi.org/10.1359/jbmr.1998.13.3.500>.
- Berry, Diane J., Kathryn Hesketh, Chris Power, and Elina Hyppönen. 2011. "Vitamin D Status Has a Linear Association with Seasonal Infections and Lung Function in British Adults." *British Journal of Nutrition* 106 (9): 1433–40. <https://doi.org/10.1017/S0007114511001991>.
- Bezuglov, Eduard, Maria Shoshorina, Artemii Lazarev, Anton Emanov, Egana Koroleva, Ilysyuyar Anishchenko, Zbigniew Waśkiewicz, Mikhail Butovskiy, and Ryland Morgans. 2023. "Does Vitamin D Affect Strength and Speed Characteristics and Testosterone Concentration in Elite Young Track and Field Athletes in the North European Summer?" *Nutrition Journal* 22 (March): 16. <https://doi.org/10.1186/s12937-023-00848-7>.
- Bishop, Meghan E., Alessandra Ahlmen, Jessica Rosendorf, Brandon J. Erickson, and Steven Cohen. 2021. "Bone Stress Injuries in Female Athletes." *Annals of Joint* 6 (0). <https://doi.org/10.21037/aoj.2020.04.04>.
- Brännström, André, Ji-Guo Yu, Per Jonsson, Torbjörn Akerfeldt, Mats Stridsberg, and Michael Svensson. 2016. "Vitamin D in Relation to Bone Health and Muscle Function in Young Female Soccer Players." *European Journal of Sport Science* 17 (September): 1–8. <https://doi.org/10.1080/17461391.2016.1225823>.
- Chen, Yilin, Minhoo Kim, Sanjana Paye, and Bérénice A. Benayoun. 2022. "Sex as a Biological Variable in Nutrition Research: From Human Studies to Animal Models." *Annual Review of Nutrition* 42 (Volume 42, 2022): 227–50. <https://doi.org/10.1146/annurev-nutr-062220-105852>.
- Chu, Chang, Oleg Tsuprykov, Xin Chen, Saban Elitok, Bernhard K. Krämer, and Berthold Hochoer. 2021. "Relationship Between Vitamin D and Hormones Important for Human Fertility in Reproductive-Aged Women." *Frontiers in Endocrinology* 12 (April). <https://doi.org/10.3389/fendo.2021.666687>.
- Close, G. L., J. Russell, J. N. Cobley, D. J. Owens, G. Wilson, W. Gregson, W. D. Fraser, and J. P. Morton. 2012. "Assessment of Vitamin D Concentration in Non-Supplemented Professional Athletes and Healthy

- Adults During the Winter Months in the UK: Implications for Skeletal Muscle Function.” *Journal of Sports Sciences* 31 (4): 344–53. <https://doi.org/10.1080/02640414.2012.733822>.
- Close, Graeme L., Jill Leckey, Marcelle Patterson, Warren Bradley, Daniel J. Owens, William D. Fraser, and James P. Morton. 2013. “The Effects of Vitamin D(3) Supplementation on Serum Total 25[OH]D Concentration and Physical Performance: A Randomised Dose-Response Study.” *British Journal of Sports Medicine* 47 (11): 692–96. <https://doi.org/10.1136/bjsports-2012-091735>.
- Cowley, Emma S., Alyssa A. Olenick, Kelly L. McNulty, and Emma Z. Ross. 2021. “‘Invisible Sportswomen’: The Sex Data Gap in Sport and Exercise Science Research.” *Women in Sport and Physical Activity Journal* 29 (2): 146–51. <https://doi.org/10.1123/wspaj.2021-0028>.
- Dubuc, G. R., S. D. Phinney, J. S. Stern, and P. J. Havel. 1998. “Changes of Serum Leptin and Endocrine and Metabolic Parameters After 7 Days of Energy Restriction in Men and Women.” *Metabolism: Clinical and Experimental* 47 (4): 429–34. [https://doi.org/10.1016/s0026-0495\(98\)90055-5](https://doi.org/10.1016/s0026-0495(98)90055-5).
- Elliott-Sale, Kirsty J., Clare L. Minahan, Xanne A. K. Janse de Jonge, Kathryn E. Ackerman, Sarianna Sipilä, Naama W. Constantini, Constance M. Lebrun, and Anthony C. Hackney. 2021. “Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women.” *Sports Medicine (Auckland, N.Z.)* 51 (5): 843–61. <https://doi.org/10.1007/s40279-021-01435-8>.
- Emmonds, Stacey, Omar Heyward, and Ben Jones. 2019. “The Challenge of Applying and Undertaking Research in Female Sport.” *Sports Medicine - Open* 5 (1): 51. <https://doi.org/10.1186/s40798-019-0224-x>.
- Goolsby, Marci A., and Nicole Boniquit. 2017. “Bone Health in Athletes.” *Sports Health* 9 (2): 108–17. <https://doi.org/10.1177/1941738116677732>.
- Gruodytė, R., J. Jürimäe, A. Cicchella, C. Stefanelli, C. Passariello, and T. Jürimäe. 2010. “Adipocytokines and Bone Mineral Density in Adolescent Female Athletes.” *Acta Paediatrica (Oslo, Norway: 1992)* 99 (12): 1879–84. <https://doi.org/10.1111/j.1651-2227.2010.01905.x>.
- Hamilton, B. 2010. “Vitamin D and Human Skeletal Muscle.” *Scandinavian Journal of Medicine & Science in Sports* 20 (2): 182–90. <https://doi.org/10.1111/j.1600-0838.2009.01016.x>.
- Heikura, Ida A., Arja L. T. Uusitalo, Trent Stellingwerff, Dan Bergland, Antti A. Mero, and Louise M. Burke. 2018. “Low Energy Availability Is Difficult to Assess but Outcomes Have Large Impact on Bone Injury Rates in Elite Distance Athletes.” *International Journal of Sport Nutrition and Exercise Metabolism* 28 (4): 403–11. <https://doi.org/10.1123/ijnsnem.2017-0313>.
- Hickey, M. S., J. A. Houmard, R. V. Considine, G. L. Tyndall, J. B. Midgette, K. E. Gavigan, M. L. Weidner, M. R. McCammon, R. G. Israel, and J. F. Caro. 1997. “Gender-Dependent Effects of Exercise Training on Serum Leptin Levels in Humans.” *American Journal of Physiology-Endocrinology and Metabolism* 272 (4): E562–66. <https://doi.org/10.1152/ajpendo.1997.272.4.E562>.
- Holick, Michael F. 2007. “Vitamin D Deficiency.” *The New England Journal of Medicine* 357 (3): 266–81. <https://doi.org/10.1056/NEJMr070553>.
- Hutchins, Kate P., David N. Borg, Aaron J. E. Bach, Joshua J. Bon, Geoffrey M. Minett, and Ian B. Stewart. 2021. “Female (Under) Representation in Exercise Thermoregulation Research.” *Sports Medicine - Open* 7 (1): 43. <https://doi.org/10.1186/s40798-021-00334-6>.
- Ihle, Rayan, and Anne B. Loucks. 2004. “Dose-Response Relationships Between Energy Availability and Bone Turnover in Young Exercising Women.” *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research* 19 (8): 1231–40. <https://doi.org/10.1359/JBMR.040410>.
- Jagim, Andrew, Margaret Jones, Andrew Askow, Joel Luedke, Jacob Erickson, Jennifer Fields, and Chad Kerkick. 2023. “Sex Differences in Resting Metabolic Rate Among Athletes and Association with Body Composition Parameters: A Follow-Up Investigation.” *Faculty Scholarship* 8 (3). <https://doi.org/https://doi.org/10.3390/jfink8030109>.
- Jakobsen, Marie Munk, Rie Harboe Nygaard, Johanne Andersen Hojbjerg, and Julie Brogaard Larsen. 2021. “The Association Between Vitamin D Status and Overuse Sport Injuries: A Systematic Review and Meta-Analysis.” *TRANSLATIONAL SPORTS MEDICINE* 4 (5): 553–64. <https://doi.org/10.1002/tsm2.269>.
- Jakše, Boštjan, Barbara Jakše, Nataša Fidler Mis, Borut Jug, Dorica Šajber, Uroš Godnov, and Ivan Čuk. 2021. “Nutritional Status and Cardiovascular Health in Female Adolescent Elite-Level Artistic Gymnasts and Swimmers: A Cross-Sectional Study of 31 Athletes.” *Journal of Nutrition and Metabolism* 2021

- (January): 8810548. <https://doi.org/10.1155/2021/8810548>.
- Jastrzębska, Maria, Mariusz Kaczmarczyk, and Zbigniew Jastrzębski. 2016. "Effect of Vitamin D Supplementation on Training Adaptation in Well-Trained Soccer Players." *The Journal of Strength & Conditioning Research* 30 (9): 2648. <https://doi.org/10.1519/JSC.0000000000001337>.
- Jung, Hyun Chul, Myong Won Seo, Sukho Lee, Sung Woo Jung, and Jong Kook Song. 2018. "Correcting Vitamin D Insufficiency Improves Some But Not All Aspects of Physical Performance During Winter Training in Taekwondo Athletes." *International Journal of Sport Nutrition and Exercise Metabolism* 28 (6): 635–43. <https://doi.org/10.1123/ijsnem.2017-0412>.
- Jürimäe, Jaak, Rita Gruodyte-Racienė, and Adam D. G. Baxter-Jones. 2018. "Effects of Gymnastics Activities on Bone Accrual During Growth: A Systematic Review." *Journal of Sports Science & Medicine* 17 (2): 245–58. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5950742/>.
- Kirchner, E. M., R. D. Lewis, and P. J. O'Connor. 1996. "Effect of Past Gymnastics Participation on Adult Bone Mass." *Journal of Applied Physiology* 80 (1): 226–32. <https://doi.org/10.1152/jappl.1996.80.1.226>.
- Knechtle, Beat, Zbigniew Jastrzębski, Lee Hill, and Pantelis T. Nikolaidis. 2021. "Vitamin D and Stress Fractures in Sport: Preventive and Therapeutic Measures—A Narrative Review." *Medicina* 57 (3): 223. <https://doi.org/10.3390/medicina57030223>.
- Kudlac, J., D. L. Nichols, C. F. Sanborn, and N. M. DiMarco. 2004. "Impact of Detraining on Bone Loss in Former Collegiate Female Gymnasts." *Calcified Tissue International* 75 (6): 482–87. <https://doi.org/10.1007/s00223-004-0228-4>.
- Lappe, Joan, Diane Cullen, Gleb Haynatzki, Robert Recker, Renee Ahlf, and Kerry Thompson. 2008. "Calcium and Vitamin d Supplementation Decreases Incidence of Stress Fractures in Female Navy Recruits." *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research* 23 (5): 741–49. <https://doi.org/10.1359/jbmr.080102>.
- Larson-Meyer, D. Enette, and Kentz S. Willis. 2010. "Vitamin D and Athletes." *Current Sports Medicine Reports* 9 (4): 220–26. <https://doi.org/10.1249/JSR.0b013e3181e7dd45>.
- Larson-Meyer, Enette. 2015. "The Importance of Vitamin D for Athletes." *Gatorade Sports Science Institute*. <http://www.gssiweb.org:80/en/sports-science-exchange/article/sse-148-the-importance-of-vitamin-d-for-athletes>.
- Lerchbaum, Elisabeth, and Barbara Obermayer-Pietsch. 2012. "Vitamin D and Fertility: A Systematic Review." *European Journal of Endocrinology* 166 (5): 765–78. <https://doi.org/10.1530/EJE-11-0984>.
- Löfberg, Ida E., Jari E. Karppinen, Vesa Laatikainen-Raussi, Maarit Lehti, Anthony C. Hackney, Johanna K. Ihalainen, and Ritva S. Mikkonen. 2024. "Resting Energy Expenditure, Metabolic and Sex Hormones in Two Phases of the Menstrual and Hormonal Contraceptive Cycles." *Medicine and Science in Sports and Exercise*, August. <https://doi.org/10.1249/MSS.00000000000003518>.
- Logue, Danielle M., Sharon M. Madigan, Anna Melin, Eamonn Delahunt, Mirjam Heinen, Sarah-Jane Mc Donnell, and Clare A. Corish. 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): 835. <https://doi.org/10.3390/nu12030835>.
- Loucks, A. B., and E. M. Heath. 1994. "Induction of Low-T3 Syndrome in Exercising Women Occurs at a Threshold of Energy Availability." *The American Journal of Physiology* 266 (3 Pt 2): R817–823. <https://doi.org/10.1152/ajpregu.1994.266.3.R817>.
- Lundsgaard, Anne-Marie, and Bente Kiens. 2014. "Gender Differences in Skeletal Muscle Substrate Metabolism – Molecular Mechanisms and Insulin Sensitivity." *Frontiers in Endocrinology* 5 (November): 195. <https://doi.org/10.3389/fendo.2014.00195>.
- Macdonald, H. M., A. Mavroei, W. D. Fraser, A. L. Darling, A. J. Black, L. Aucott, F. O'Neill, et al. 2011. "Sunlight and Dietary Contributions to the Seasonal Vitamin D Status of Cohorts of Healthy Postmenopausal Women Living at Northerly Latitudes: A Major Cause for Concern?" *Osteoporosis International: A Journal Established as Result of Cooperation Between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 22 (9): 2461–72. <https://doi.org/10.1007/s00198-010-1467-z>.
- Mair, Kirsty M., Rosemary Gaw, and Margaret R. MacLean. 2020. "Obesity, Estrogens and Adipose Tissue Dysfunction – Implications for Pulmonary Arterial Hypertension." *Pulmonary Circulation* 10 (3): 2045894020952019. <https://doi.org/10.1177/2045894020952023>.
- Maya, Jacqueline, and Madhusmita Misra. 2022. "The Female Athlete Triad: Review of Current Literature."

- Current Opinion in Endocrinology, Diabetes, and Obesity* 29 (1): 44–51. <https://doi.org/10.1097/MED.0000000000000690>.
- McBryde, A. M. 1985. “Stress Fractures in Runners.” *Clinics in Sports Medicine* 4 (4): 737–52.
- McKay, Alannah K. A., Trent Stellingwerff, Ella S. Smith, David T. Martin, Iñigo Mujika, Vicky L. Goosey-Tolfrey, Jeremy Sheppard, and Louise M. Burke. 2021. “Defining Training and Performance Caliber: A Participant Classification Framework.” *International Journal of Sports Physiology and Performance* 17 (2): 317–31. <https://doi.org/10.1123/ijsp.2021-0451>.
- Melin, Anna, Asa B. Tornberg, Sven Skouby, Jens Faber, Christian Ritz, Anders Sjödin, and Jorunn Sundgot-Borgen. 2014. “The LEAF Questionnaire: A Screening Tool for the Identification of Female Athletes at Risk for the Female Athlete Triad.” *British Journal of Sports Medicine* 48 (7): 540–45. <https://doi.org/10.1136/bjsports-2013-093240>.
- Millward, David, Allison D. Root, Jeremy Dubois, Randall P. Cohen, Luis Valdivia, Bruce Helming, Justin Kokoskie, Anna L. Waterbrook, and Stephen Paul. 2020. “Association of Serum Vitamin D Levels and Stress Fractures in Collegiate Athletes.” *Orthopaedic Journal of Sports Medicine* 8 (12): 2325967120966967. <https://doi.org/10.1177/2325967120966967>.
- Moreira, Carolina A., and John P. Bilezikian. 2017. “Stress Fractures: Concepts and Therapeutics.” *The Journal of Clinical Endocrinology and Metabolism* 102 (2): 525–34. <https://doi.org/10.1210/jc.2016-2720>.
- Morton, James P., Zafar Iqbal, Barry Drust, Darren Burgess, Graeme L. Close, and Peter D. Brukner. 2012. “Seasonal Variation in Vitamin D Status in Professional Soccer Players of the English Premier League.” *Applied Physiology, Nutrition, and Metabolism* 37 (4): 798–802. <https://doi.org/10.1139/h2012-037>.
- Moto, Kuniko, Mika Goshozono, Suguru Torii, Akira Namba, and Motoko Taguchi. 2022. “Resting Energy Expenditure Is Lower in Japanese Female Athletes with Menstrual Disorders Than in Eumenorrheic Athletes.” *The Journal of Physical Fitness and Sports Medicine* 11 (1): 35–42. <https://doi.org/10.7600/jpfsm.11.35>.
- Murphy, Chaise, Laura D. D. Bilek, and Karsten Koehler. 2021. “Low Energy Availability with and Without a High-Protein Diet Suppresses Bone Formation and Increases Bone Resorption in Men: A Randomized Controlled Pilot Study.” *Nutrients* 13 (3): 802. <https://doi.org/10.3390/nu13030802>.
- Nana, Alisa, Gary J. Slater, Arthur D. Stewart, and Louise M. Burke. 2015. “Methodology Review: Using Dual-Energy X-Ray Absorptiometry (DXA) for the Assessment of Body Composition in Athletes and Active People.” *International Journal of Sport Nutrition and Exercise Metabolism* 25 (2): 198–215. <https://doi.org/10.1123/ijsnem.2013-0228>.
- Narla, Radhika R., and Susan M. Ott. 2018. “Bones and the Sex Hormones.” *Kidney International* 94 (2): 239–42. <https://doi.org/10.1016/j.kint.2018.03.021>.
- Neal, Sara, Jeannie Sykes, Michael Rigby, and Bryan Hess. 2015. “A Review and Clinical Summary of Vitamin D in Regard to Bone Health and Athletic Performance.” *The Physician and Sportsmedicine* 43 (2): 161–68. <https://doi.org/10.1080/00913847.2015.1020248>.
- Owens, Daniel J., Richard Allison, and Graeme L. Close. 2018. “Vitamin D and the Athlete: Current Perspectives and New Challenges.” *Sports Medicine (Auckland, N.z.)* 48 (Suppl 1): 3–16. <https://doi.org/10.1007/s40279-017-0841-9>.
- Papageorgiou, Maria, Kirsty J. Elliott-Sale, Alan Parsons, Johnathan C. Y. Tang, Julie P. Greeves, William D. Fraser, and Craig Sale. 2017. “Effects of Reduced Energy Availability on Bone Metabolism in Women and Men.” *Bone* 105 (December): 191–99. <https://doi.org/10.1016/j.bone.2017.08.019>.
- Papageorgiou, Maria, Daniel Martin, Hannah Colgan, Simon Cooper, Julie P. Greeves, Jonathan C. Y. Tang, William D. Fraser, Kirsty J. Elliott-Sale, and Craig Sale. 2018. “Bone Metabolic Responses to Low Energy Availability Achieved by Diet or Exercise in Active Eumenorrheic Women.” *Bone* 114 (September): 181–88. <https://doi.org/10.1016/j.bone.2018.06.016>.
- Park, Hyeon-Kyu, and Rexford S. Ahima. 2015. “Physiology of Leptin: Energy Homeostasis, Neuroendocrine Function and Metabolism.” *Metabolism: Clinical and Experimental* 64 (1): 24–34. <https://doi.org/10.1016/j.metabol.2014.08.004>.
- Ribbans, William J., Randeep Aujla, Seamus Dalton, and James A. Nunley. 2021. “Vitamin D and the Athlete-Patient: State of the Art.” *Journal of ISAKOS: Joint Disorders & Orthopaedic Sports Medicine* 6 (1): 46–60. <https://doi.org/10.1136/jisakos-2020-000435>.
- Rockwell, Michelle, Madlyn Frisard, Janet Rankin, Jennifer Zabinsky, Ryan Mcmillan, Wen You, Kevin Davy, and Matthew Hulver. 2020. “Effects of Seasonal Vitamin D3 Supplementation on Strength,

- Power, and Body Composition in College Swimmers.” *International Journal of Sport Nutrition and Exercise Metabolism* 30: 1–9. <https://doi.org/10.1123/ijsnem.2019-0250>.
- Rogers, Margot A., Michael K. Drew, Renee Appaneal, Greg Lovell, Bronwen Lundy, David Hughes, Nicole Vlahovich, Gordon Waddington, and Louise M. Burke. 2021. “The Utility of the Low Energy Availability in Females Questionnaire to Detect Markers Consistent With Low Energy Availability-Related Conditions in a Mixed-Sport Cohort.” *International Journal of Sport Nutrition and Exercise Metabolism* 31 (5): 427–37. <https://doi.org/10.1123/ijsnem.2020-0233>.
- Rugvedh, Padigela, Ppavani Gundreddy, and Bhushan Wandile. 2023. “The Menstrual Cycle’s Influence on Sleep Duration and Cardiovascular Health: A Comprehensive Review.” *Cureus* 15 (10): e47292. <https://doi.org/10.7759/cureus.47292>.
- Sale, Craig, and Kirsty Jayne Elliott-Sale. 2019. “Nutrition and Athlete Bone Health.” *Sports Medicine (Auckland, N.Z.)* 49 (Suppl 2): 139–51. <https://doi.org/10.1007/s40279-019-01161-2>.
- Sanborn, C. F., and C. M. Jankowski. 1994. “Physiologic Considerations for Women in Sport.” *Clinics in Sports Medicine* 13 (2): 315–27.
- Santos, Livia, Kirsty Jayne Elliott-Sale, and Craig Sale. 2017. “Exercise and Bone Health Across the Lifespan.” *Biogerontology* 18 (6): 931–46. <https://doi.org/10.1007/s10522-017-9732-6>.
- Sienkiewicz, Elizabeth, Faidon Magkos, Konstantinos N. Aronis, Mary Brinkoetter, John P. Chamberland, Sharon Chou, Kalliopi M. Arampatzi, Chuanyun Gao, Anastasia Koniaris, and Christos S. Mantzoros. 2011. “Long-Term Metreleptin Treatment Increases Bone Mineral Density and Content at the Lumbar Spine of Lean Hypoleptinemic Women.” *Metabolism: Clinical and Experimental* 60 (9): 1211–21. <https://doi.org/10.1016/j.metabol.2011.05.016>.
- Sim, Alexiaa, and Stephen F. Burns. 2021. “Review: Questionnaires as Measures for Low Energy Availability (LEA) and Relative Energy Deficiency in Sport (RED-S) in Athletes.” *Journal of Eating Disorders* 9 (1): 41. <https://doi.org/10.1186/s40337-021-00396-7>.
- Skarakis, Nikitas S., George Mastorakos, Neoklis Georgopoulos, and Dimitrios G. Goulis. 2021. “Energy Deficiency, Menstrual Disorders, and Low Bone Mineral Density in Female Athletes: A Systematic Review.” *Hormones (Athens, Greece)* 20 (3): 439–48. <https://doi.org/10.1007/s42000-021-00288-0>.
- Smith, Ella S., Alannah K. A. McKay, Megan Kuikman, Kathryn E. Ackerman, Rachel Harris, Kirsty J. Elliott-Sale, Trent Stellingwerff, and Louise M. Burke. 2022. “Managing Female Athlete Health: Auditing the Representation of Female Versus Male Participants Among Research in Supplements to Manage Diagnosed Micronutrient Issues.” *Nutrients* 14 (16): 3372. <https://doi.org/10.3390/nu14163372>.
- Souza, Mary Jane De, Aurelia Nattiv, Elizabeth Joy, Madhusmita Misra, Nancy I. Williams, Rebecca J. Mallinson, Jenna C. Gibbs, et al. 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): 289–89. <https://doi.org/10.1136/bjsports-2013-093218>.
- Stevenson, John. 2023. “Prevention and Treatment of Osteoporosis in Women.” *Post Reproductive Health* 29 (1): 11–14. <https://doi.org/10.1177/20533691221139902>.
- Tenforde, Adam S., Lauren C. Sayres, Kristin L. Sainani, and Michael Fredericson. 2010. “Evaluating the Relationship of Calcium and Vitamin D in the Prevention of Stress Fracture Injuries in the Young Athlete: A Review of the Literature.” *PM & R: The Journal of Injury, Function, and Rehabilitation* 2 (10): 945–49. <https://doi.org/10.1016/j.pmrj.2010.05.006>.
- Thomas, T., B. Burguera, L. J. Melton, E. J. Atkinson, W. M. O’Fallon, B. L. Riggs, and S. Khosla. 2001. “Role of Serum Leptin, Insulin, and Estrogen Levels as Potential Mediators of the Relationship Between Fat Mass and Bone Mineral Density in Men Versus Women.” *Bone* 29 (2): 114–20. [https://doi.org/10.1016/s8756-3282\(01\)00487-2](https://doi.org/10.1016/s8756-3282(01)00487-2).
- Thompson, Frances E., and Amy F. Subar. 2017. “Dietary Assessment Methodology.” In *Nutrition in the Prevention and Treatment of Disease*, 5–48. Elsevier. <https://doi.org/10.1016/B978-0-12-802928-2.00001-1>.
- Todd, Joshua J., Emeir M. McSorley, L. Kirsty Pourshahidi, Sharon M. Madigan, Eamon Laird, Martin Healy, and Pamela J. Magee. 2017. “Vitamin D3 Supplementation Using an Oral Spray Solution Resolves Deficiency but Has No Effect on VO2 Max in Gaelic Footballers: Results from a Randomised, Double-Blind, Placebo-Controlled Trial.” *European Journal of Nutrition* 56 (4): 1577. <https://doi.org/10.1007/s00394-016-1202-4>.

- Upadhyay, Jagriti, Olivia M. Farr, and Christos S. Mantzoros. 2015. "The Role of Leptin in Regulating Bone Metabolism." *Metabolism: Clinical and Experimental* 64 (1): 105–13. <https://doi.org/10.1016/j.metabol.2014.10.021>.
- Wilson-Barnes, Saskia L., Julie E. A. Hunt, Emma L. Williams, Sarah J. Allison, James J. Wild, Joe Wainwright, Susan A. Lanham-New, and Ralph J. F. Manders. 2020. "Seasonal Variation in Vitamin D Status, Bone Health and Athletic Performance in Competitive University Student Athletes: A Longitudinal Study." *Journal of Nutritional Science* 9: e8. <https://doi.org/10.1017/jns.2020.1>.
- Witkoś, Joanna, Grzegorz Błażejowski, and Marcin Gierach. 2023. "The Low Energy Availability in Females Questionnaire (LEAF-Q) as a Useful Tool to Identify Female Triathletes at Risk for Menstrual Disorders Related to Low Energy Availability." *Nutrients* 15 (3): 650. <https://doi.org/10.3390/nu15030650>.
- Wyon, Matthew A., Roger Wolman, Nicolas Kolokythas, Karen Sheriff, Shaun Galloway, and Adam Mattiussi. 2019. "Effect of Vitamin D on Muscle Function and Injury in Elite Adolescent Dancers: A Randomized Double-Blind Study." *International Journal of Sports Physiology and Performance* 14 (1): 55–59. <https://doi.org/10.1123/ijsp.2018-0084>.
- Wyon, Matthew A., Roger Wolman, Alan M. Nevill, Ross Cloak, George S. Metsios, Douglas Gould, Andrew Ingham, and Yiannis Koutedakis. 2016. "Acute Effects of Vitamin D3 Supplementation on Muscle Strength in Judoka Athletes: A Randomized Placebo-Controlled, Double-Blind Trial." *Clinical Journal of Sport Medicine: Official Journal of the Canadian Academy of Sport Medicine* 26 (4): 279–84. <https://doi.org/10.1097/JSM.0000000000000264>.
- Yagüe, Mirian de la Puente, Luis Collado Yurrita, Maria J. Ciudad Cabañas, and Marioa A. Cuadrado Cenual. 2020. "Role of Vitamin D in Athletes and Their Performance: Current Concepts and New Trends." *Nutrients* 12 (2). <https://doi.org/10.3390/nu12020579>.