Nutrition factors associated with rib stress injury in elite rowers

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Nutrition Factors Associated with Rib Stress Injury in Elite Rowers

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Declaration of Authorship & Sources

This thesis contains no material that has been extracted in whole or in part from a thesis that I have submitted towards the award of any other degree or diploma in any other tertiary institution.
No other person's work has been used without due acknowledgement in the main text of the thesis.
All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees.

Bronwen Lundy, 7th April 2022

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Life outside of the PhD has been huge; bushfires, floods, home schooling, two Olympic Games, world championships, world cups and more covid tests than I wish to count. Along the way I lost my

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Contents

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Abbreviations

ACTH	Adrenocorticotropic hormone
AMS	Aging Male Symptom questionnaire
AN	Anorexia nervosa
BAP	bone specific alkaline phosphatase
BGL	Blood glucose level
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Areal Bone mineral density
BPAQ	Bone-specific physical activity questionnaire
BTM	Bone turnover markers
BTR	Bone turnover ratio (P1NP/ β-CTX-I)
cOC	Carboxylated osteocalcin
CAL	Calcium
CON	Control
CHO	carbohydrate
CTX-II	C-telopeptide of type II collagen
DHEA-S	Dehydroepiandrosterone sulphate
DXA	Dual X-ray Absorptiometry
EA	Energy availability
EB	Energy balance
EDE-Q	Eating disorder examination questionnaire
EDI	Eating disorder inventory
EEE	Exercise energy expenditure
EHMC	Exercise Hypogonadal Male Condition
EI	Energy Intake
F	Female
FAT CRA	Female Athlete Triad Cumulative Risk Assessment
FBC	Full blood count
FFA	Free fatty acids
FFM	Fat free mass
FFQ	Food frequency questionnaire
FSH	Follicle stimulating hormone
FTCR	Free testosterone to cortisol ratio

FTES	Free testosterone
GH	Growth hormone
GNRH	Gonadotrophin releasing hormone
iCa	Serum ionized calcium
IGF-1	Insulin like growth factor
IL-6	Interleukin 6
IV	Intravenous
LBM	Lean body mass
LCHF	Low carbohydrate high fat
LEA	Low energy availability
LEAF-Q	Low energy availability in female athletes questionnaire
LH	Luteinising hormone
M	Male
MAT	Male athlete triad
MBCQ	Male body checking questionnaire
NEAT	Non exercise activity thermogenesis
NTx	Cross linked n-telopeptide of type 1 collagen
P1CP	Procollagen type 1 C terminal propeptide
P1NP	Procollagen type 1 N terminal propeptide
POMS	Profile of Mood States
PTH	Parathyroid hormone
PYD	Pyridium cross-links
RED-S	Relative energy deficiency in sport
RED-S CAT	Relative energy deficiency in sport clinical assessment tool
REE	Resting energy expenditure
RMR	Resting metabolic rate
RMR_{ratio}	Measured to predicted RMR
RSI	Rib stress injury
SHBG	Sex hormone binding globulin
T_3	Free triiodothyronine
T_4	Thyroxine
TES	Total Testosterone
TG	triglycerides
tOC	Total osteocalcin
TRAP 5b	Tartrate-resistant acid phosphatase 5b

TSH	Thyroid stimulating hormone
TTCR	Total testosterone to cortisol ratio
ucOC	Undercarboxylated osteocalcin
VO_{2max}	Maximal oxygen consumption
VO_{2peak}	Peak oxygen uptake
VT	Ventilatory threshold
β-CTX-I	C-terminal telopeptide of type I collagen
WNL	Within normal limits

Rowing Definitions

Rowing (still water)	This is a sport contested locally, nationally and internationally and is included in the summer Olympic programme. Races are held over a 2km course, are intense and take approximately 5-8 minutes to cover the distance. There are two disciplines in rowing – sweep and sculling and rowers may compete as an individual or team of two, four or eight individuals. There are two weight categories- lightweight and openweight.
Sweep	Where rowers have one oar each in pairs with one oar on each side of the boat. Sweep rowers will row in a pair, four or eight.
Sculling	Sculling involves rowing with two oars each, one on each side of the boat. Scullers will row in a single, double or quad.
Lightweight	Lightweight rowing is a category of rowing where limits are placed on the maximum body weight of competitors with the intention of encouraging accessibility of the sport to nations with smaller stature. Lightweight males can be up to 72.5kg with a crew average weight of 70kg and females up to 59kg with a crew average of 57kg. There is currently debate as to whether lightweight rowing will be continued in the Olympic programme.
Openweight	This is a category within rowing where there are no weight limits. Athletes within this category are often very tall (>190cm for males and >175cm for females).

Publications List

Publications Relating to the Thesis

1. **Lundy, B.**, Suni, V., Drew, M., Trease, L and Burke, L. M. Nutrition factors associated with rib stress injury history in elite rowers. Journal of Science and Medicine in Sport (2022). Aug 31: S1440-2440 (22)00246-8. doi: 10.1016/j.jsams.2022.08.017, online ahead of print.

2. **Lundy, B.**, Torstveit, M. K., Stenqvist, T. B., Burke, L. M., Garthe, I., Slater, G., Ritz, C., Melin, A. K., Screening for low energy availability in male athletes: attempted validation of LEAM-Q, Nutrients (2022) Apr 29: 14(9):1873. doi: 10.3390/nu14091873.

3. **Lundy, B.**, McKay, A. K. A, Fensham, N. C., Tee, N., Anderson, B., Morabito, A., Ross, M. L. R., Burke. L. M. The Impact of Acute Calcium Intake on Bone Turnover Markers During a Training Day in Elite Rowers, *Medicine and Science in Sport and Exercise* (2022), Aug 12 doi: 10.1249/MSS.0000000000003022. Online ahead of print.

Additional Publications During the Candidature

2017 Drew, M. K., Vlahovich, N., Hughes, D., Appaneal, R., Peterson, K., Burke, L., **Lundy, B.**, Toomey, M., Watts, D., Lovell, G., Praet, S., Halson, S., Colbey, C., Manzanero, S., Welvaert, M., West, N., Pyne, D. B. and Waddington, G. A Multifactorial evaluation of illness risk factors in athletes preparing for the Summer Olympic Games. *J Sci Med Sport*. 2017;20(8):745-50

2017 Woods AL, Garvican-Lewis LA, **Lundy B**, Rice AJ, Thompson KG. New approaches to determine fatigue in elite athletes during intensified training: Resting metabolic rate and pacing profile. *PloS one*. 2017;12(3): e0173807.

2018 Burke LM, Close GL, **Lundy B**, Mooses M, Morton JP, Tenforde AS. Relative Energy Deficiency in Sport in Male Athletes: A Commentary on Its Presentation Among Selected Groups of Male Athletes. *Int J Sport Nutr Exerc Metab.* 2018;28(4):364-74.

2018 Burke LM, **Lundy B**, Fahrenholtz IL, Melin AK. Pitfalls of Conducting and Interpreting Estimates of Energy Availability in Free-Living Athletes. *Int J Sport Nutr Exerc Metab.* 2018;28(4):350-63.

2018 Mountjoy, M., Sundgot-Borgen, J., Burke, L., Ackerman, K. E., Blauwet, C., Constantini, N., Lebrun, C., **Lundy, B.**, Melin, A., Meyer, N., Sherman, R., Tenforde, A. S., Torstveit, M. K. and Budgett, R. International Olympic Committee (IOC) Consensus Statement on Relative Energy Deficiency in Sport (RED-S): 2018 Update. *Int J Sport Nutr Exerc Metab.* 2018;28(4):316-31.

2018 Woods AL, Rice AJ, Garvican-Lewis LA, Wallett AM, **Lundy B**, Rogers MA, et al. The effects of intensified training on resting metabolic rate (RMR), body composition and performance in trained cyclists. *PloS one.* 2018;13(2): e0191644.

2018 Drew M, Vlahovich N, Hughes D, Appaneal R, Burke LM, **Lundy B**, Rogers M, Toomey M, Watts D, Lovell G, Praet S, Halson SL, Colbey C, Manzanero S, Welvaert M, West NP, Pyne DB, Waddington G. Prevalence of illness, poor mental health and sleep quality and low energy availability prior to the 2016 Summer Olympic Games. *Br J Sports Med*. 2018 Jan;52(1):47-53

2020 Larsen, B., Cox, A., Colbey, C., Drew, M., McGuire, H., Fazekas de St Groth, B., Hughes, D., Vlahovich, N., Waddington, G., Burke, L., **Lundy, B**., West, N. and Minahan, C. Inflammation and Oral Contraceptive Use in Female Athletes Before the Rio Olympic Games. *Frontiers in physiology*. 2020;11:497.

2020 Larsen, B., Cox, A., Colbey, C., Drew, M., McGuire, H., Fazekas de St Groth, B., Hughes, D., Vlahovich, N., Waddington, G., Burke, L., **Lundy, B.**, West, N. and Minahan, C. Key viral immune genes and pathways identify elite athletes with URS. *Exercise immunology review*. 2020;26:56-78

2021 Fensham, N., McKay, A. K. A., Tee, N., **Lundy, B.**, Anderson, B., Morabito, A., Ross, M., Burke, L. M. Sequential submaximal training in elite male rowers does not result in amplified increases on Interleukin-6 or Hepcidin. *Int J Sport Nutr Exerc Metab.* Epub ahead of print, DOI: https://doi.org/10.1123/ijsnem.2021-0263

2021 Rogers MA, Drew MK, Appaneal R, Lovell G, **Lundy B**, Hughes D, Vlahovich, N., Waddington, G. and Burke, L. M. 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. *Int J Sport Nutr Exerc Metab.* 2021:1-11.

Abstract

Rib stress injuries (RSI) contribute the highest loss of training time of all rowing related injuries, negatively affecting training consistency and the ability to produce optimal performances when needed. Nutrition interacts with training to moderate bone growth, repair and maintenance and, as such, is of interest in understanding changeable contributors to injury.

Given the scarcity of available research, this thesis investigated nutrition factors associated with RSI, the development of a tool to assess one of these factors, low energy availability (LEA), and the effects of acute calcium intake on markers of bone turnover (BTM) over a typical training day.

Study 1 (Chapter 4) was a cross-sectional analysis of RSI history and related nutrition factors in elite Australian rowers (n= 133). Bone mineral density (BMD), body composition, vitamin D and K status, usual calcium intake, menstrual history, diet restriction, age, sex, training age and injury history were assessed.

Diet restriction was inversely related to spine and rib BMD. Vitamin D and K status, and calcium intake were not associated with injury. Among rowers with RSI history, lightweight males had lower total bone mass, femur and rib BMD, whereas heavyweight females had lower rib BMD. In relation to RSI history, the best models included rib, spine or femur BMD with age, body fat and sex. A female-specific model included current menstrual dysfunction, age and body fat levels.

Study 2 (Chapter 6) aimed to develop and validate a screening tool for low energy availability (LEA) in male athletes. This was a multi-centre collaboration, recruiting male athletes (n=310) from a variety of sports. Multivariate analysis was used to identify associations between variable responses and clinical markers, and Receiver Operating Characteristics (ROC) curve analysis of variables, with an inclusion threshold of 60% sensitivity. Of the variables, dizziness, illness, fatigue, and sex drive had sufficient sensitivity to be retained in the questionnaire, but only low sex drive was able to distinguish between LEA cases and controls. In this large and international cohort, low sex drive was the most effective self-reported symptom in identifying male athletes requiring further clinical assessment for LEA.

Study 3 (Chapter 8) examined the influence of acute calcium intake on bone turnover markers over a typical training day in elite male rowers. While acute exercise typically increases BTM, the impact of subsequent sessions and the interaction with pre-exercise calcium intake remains unclear despite the application to the 'real life' training of athletes.

Using a randomized crossover design, elite male rowers (n=16) completed two trials, a week apart, consisting of two 90-minute rowing ergometer sessions (Ex1, Ex2) separated by 150min. Prior to each

trial, participants consumed a high (CAL: ~1000 mg) or isocaloric low (CON: <10 mg) calcium meal. BTM (parathyroid hormone: PTH; C-terminal telopeptide of type I collagen: β-CTX-I; osteocalcin: OC) and serum ionised calcium (iCa) were monitored from baseline to 3 hours post Ex2.

While each session caused perturbances of serum iCa, CAL maintained calcium concentrations above those of CON for most time points, 4.5 and 2.4% higher post EX1 and EX2 respectively. The decrease in iCa in CON was associated with an elevation of blood PTH and β-CTX-I over this period of repeated training sessions and their recovery, particularly during and after Ex2.

Pre-exercise intake of a calcium-rich meal prior to training sessions undertaken within the same day had a cumulative and prolonged effect on the stabilisation of blood iCa during exercise. In turn, this reduced the post-exercise PTH response, potentially attenuating the increase in markers of bone resorption.

Collectively the findings of the thesis were

1. Clarification of associations between nutrition factors and RSI history informing future monitoring and interventions, LEA is important.
2. Association of rib BMD with RSI providing practical benefits to frequency of monitoring and lower radiation dose, opening avenues for better characterisation of its relationship with RSI.
3. Sex drive is an important indicator of LEA in male athletic populations
4. Pre-exercise calcium has the potential to safeguard long term bone health and reduce the risk of bone stress injuries and is a practical strategy, easily integrated into the athlete's overall sports nutrition plan, complementing those adequacy of EA.

1. Introduction

Rowing is a sport requiring a high level of endurance training consisting of between 15 to 30 hours per week and including strength, on water and cross training sessions (Fiskerstrand et al. 2004, Tran et al. 2015) and with a high energy requirement to support this training (Winkert et al. 2022). The main injuries reported tend to be those of over-use, with rib stress fractures one of the most disruptive, occurring in 8.1-16.4% of elite rowing competitors, causing significant time out of the boat and a negative effect on training consistency and competition performance (McDonnell et al. 2011). Despite the relative importance of this injury, research interests have focussed on diagnosis and management, with little attention to prevention. Indeed, it is unclear whether risk is influenced by even the most basic characteristics of the rower, such as sex, class (lightweight or heavyweight) or discipline (sweep or scull). The available research consists mostly of case studies or case series, with only a small number of cross-sectional studies investigating possible contributors. A systematic review of risk factors of rib stress fractures in rowing identified technique, bone mineral density, level of experience, sex, training load, type or intensity and changes to equipment as key risks but concluded that the current evidence is of low quality with all factors having "insufficient or conflicting evidence" (D'Ailly et al. 2016). Clinicians working with rowing athletes currently have little to guide them in appropriate prevention strategies and must draw on non-specific research from bone stress injuries in other sports or from general research into osteoporosis prevention in the elderly.

BMD has been associated with bone stress injuries across several sports but its role in rib stress injury is unclear. Calcium intake is known to important for bone health and Vitamin D for calcium absorption. Vitamin K has raised interest for a potential role in bone health however quality studies in athletic populations are lacking. LEA and menstrual dysfunction have been associated with increased bone stress injuries in athletic populations in general and are potential contributors to rib stress injuries in rowers. Methods for measuring EA are unwieldly and prone to estimation errors. While screening tools for LEA are available for female athletes, there is a lack of an equivalent for male athletic populations. A validated tool that could be used in both research and clinical settings is needed.

Pilot data suggest that elite rowers may experience a decline in BMD throughout an Olympic cycle, with greater effects observed in males than females (Lundy, unpublished data, 2016). The reasons for this decline and the differences between sex is unclear. Research conducted in cycling, another non-weight bearing exercise suggests that the acute reduction in serum ionic calcium during exercise, may stimulate resorption of bone calcium to stabilise serum calcium levels and contribute to progressive bone mineral loss. Calcium intake prior to prolonged exercise either from dietary or

supplemental sources appears to attenuate the exercise-related increase in parathyroid hormone (PTH) and its downstream effects on markers of bone resorption in non-weight bearing (cycling) and weight bearing (running) sports. This may provide a relevant strategy in elite rowing populations to attenuate bone loss. However, whether this effect is repeated when multiple training sessions are conducted on the same day, often before the training related perturbations in bone remodelling from one session have normalised, is unknown. Therefore, it is important to investigate these strategies under the real-world conditions faced by athletes.

Chapter two provides a literature review of the nutrition and physique factors that may be associated with bone stress injury in sport and ultimately how these might apply to rib stress fracture in elite rowers. It will also focus on identification of risk factors and potential prevention strategies. Additional methodological detail will be provided in chapter three and chapters four to eight include the research papers aiming to address gaps in the literature and to answer the following research questions

1. Study one: Nutrition factors associated with rib stress injury history in elite rowers
    a. What are the BMD characteristics of elite rowers?
    b. Are nutrition-related factors associated with differences in BMD in this population?
    c. What are the major dietary and related factors associated with rib stress injury history in elite rowers (e.g. calcium, vitamin D, vitamin K, diet restriction, menstrual dysfunction)?

2. Study two: Validation of a screening tool to identify risk of LEA in male athletes.

    a. What are the key biomarkers of LEA in males and how do these differ to females?

    b. Which questions are most effective at identifying male athletes at risk of LEA? How do these differ between sport types and to female athletes?

    c. Can a specific tool to identify male athletes at risk of LEA be developed?

3. Study three: Pre-training calcium supplementation and effects on bone metabolism during and after repeated bouts of rowing training in elite male rowers
    a. What is the acute effect of a typical rowing training session on serum ionic calcium and markers of bone turnover?
    b. Can calcium supplementation, via integration of calcium-rich foods in a pre-training meal, attenuate the hypothetical decline in serum ionic calcium at the onset of training and its downstream effects on markers of bone turnover in elite rowers?

c. What is the effect of multiple sessions of rowing training and the interaction of pre-exercise calcium on these parameters?

2. Literature Review

2.1 Bone Physiology

Human bone consists of the hard, dense outer coating of cortical bone with an inner core of trabecular bone. Bone undergoes a continual remodelling process of resorption and formation which is intended to respond to stressors on the bone and maintain a healthy structure. Osteoclasts breakdown bone and osteoblasts are involved in bone formation (Figure 1) (Dolan et al. 2020).

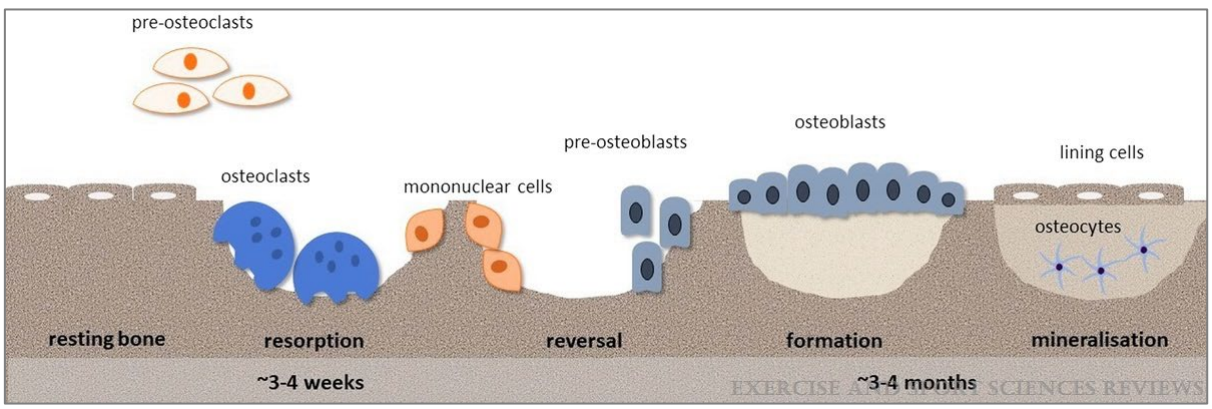


Figure 1 Bone Modelling cycle (Dolan et al. 2020)

In general, exercise interacts with this process by providing a stimulus for bone resorption and formation favouring adaptation of the bone to the increased load. Whilst exercise is frequently beneficial, it is acknowledged that there are situations where it can either exert no, or even a negative stimulus to bone (Figure 2) (Dolan et al. 2020). Whilst the optimal type and duration of exercise is not known it is thought that impact loading including vertical and multidirectional jumping or bounding is ideal (Beck et al. 2017). Rest and change of stimulus are thought to be important for keeping the bone responsive to the stimulus (Kohrt et al. 2004). Sports which provide a varied stimulus, high impact and multidirectional forces are thought to provide a better stimulus to bone formation (Heinonen et al. 1995, Burt et al. 2017) as opposed to those that are non-weight bearing such as cycling (Campion et al. 2010) and swimming (Gomez-Bruton et al. 2017) or where there are repetitive low force impacts such as distance running (Bilanin et al. 1989).

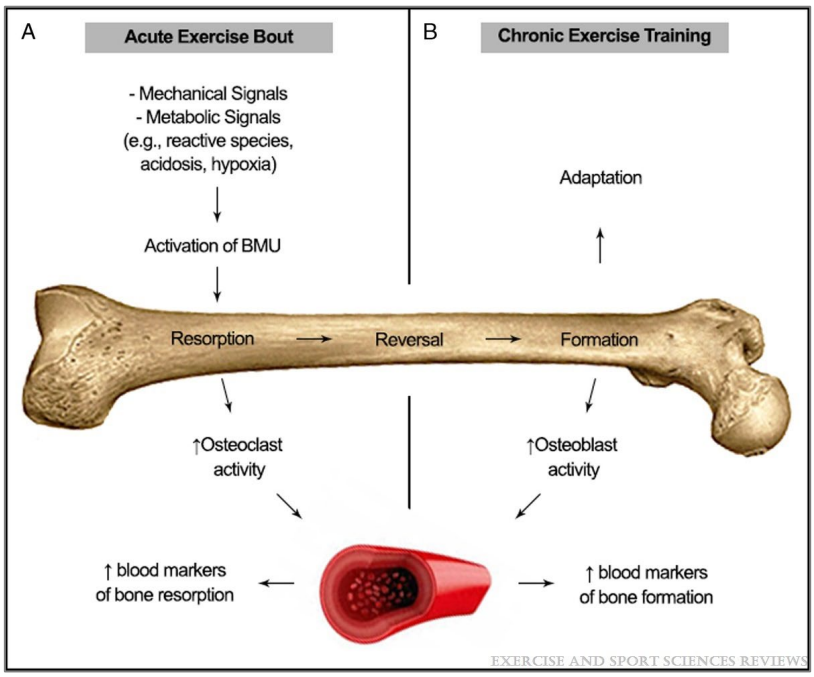


Figure 2 Bone remodelling in response to exercise (Dolan et al. 2020)

The Influence of Exercise on Bone Remodelling and the use of Bone Turnover Markers

As the bone remodelling cycle occurs over a period of months, and measurable changes in bone mass, density or microarchitecture can take years to demonstrate, it is challenging to investigate the outcomes of interventions to improve bone status (Kohrt et al. 2004, Dolan et al. 2020). The acute effect of exercise type, intensity and duration on bone are often inferred by changes in bone turnover markers, which may provide insight into the influence of chronic training on bone (Dolan et al. 2020).

BTM generally attributed as markers of bone formation include bone-specific alkaline phosphatase (BAP), Osteocalcin (OC) and Procollagen type 1 N terminal propeptide (P1NP). Markers of bone resorption include Tartrate-resistant acid phosphatase 5b (TRAP 5b), Pyridium cross-links and Collagen type I telopeptides (CTX and NTX) (See Figure 3). Variations in choice of BTM from both blood and urine samples and inconsistencies in the nomenclature of their abbreviated terms contribute to difficulties in interpreting the scientific literature regarding the influences on bone turnover or the effect of different interventions (Dolan et al. 2022).

The National Bone Health Alliance (Bauer et al. 2012, Dolan et al. 2020) propose the use of P1NP as a marker for bone formation and β-CTX-I for bone breakdown, however given (Dolan et al. 2020) P1NP is more likely to identify chronic changes in bone formation than responses to an acute exercise session and therefore other markers may be more appropriate depending on the specific study designs and exercise protocols being investigated (Dolan et al. 2020).

OC is a protein synthesised by osteoblasts and is often used as a marker for bone formation. However, OC may also be released during bone resorption and may indicate bone remodelling more than formation specifically (Ivaska et al. 2004). Reductions in OC levels have also been linked to both reduced carbohydrate intake and to LEA (Dolan et al. 2022, Fensham et al. 2022)

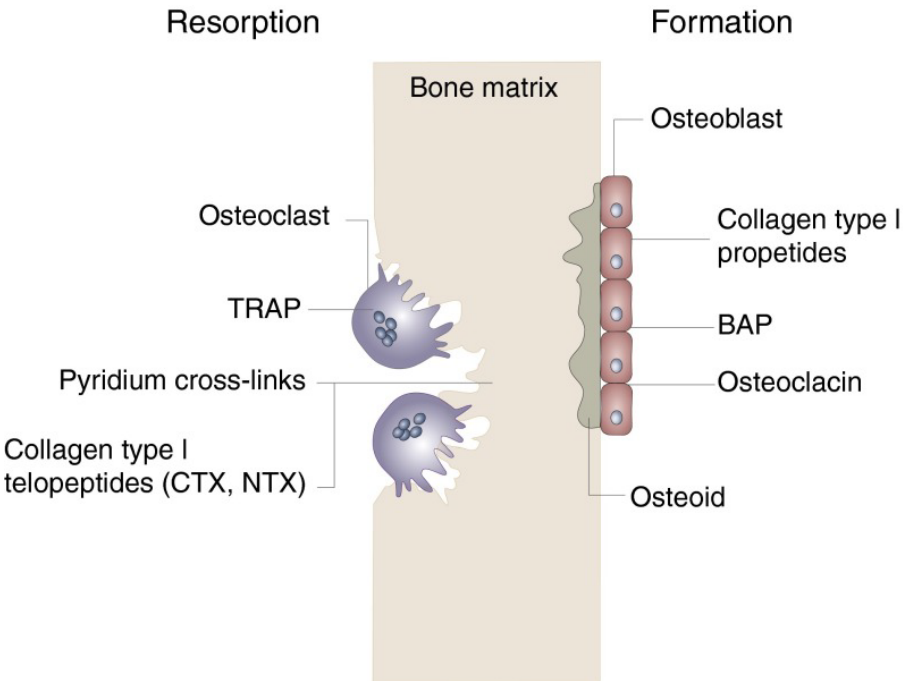


Figure 3 Bone resorption and formation and its relationship to BTM (Bonjour et al. 2014)

The primary role of parathyroid hormone (PTH) is to regulate serum calcium (iCa) levels and, as such, is often used to make inferences as to how exercise may influence bone remodelling. Falling iCa stimulates secretion of PTH and results in the breakdown of bone to release calcium and restore blood levels and are coupled with increases in β-CTX-I (Barrett et al. 1997). Changes in PTH can be difficult to interpret however, given an intermittent increase is thought to stimulates bone formation whereas prolonged continuous exposure tips the balance in favour of resorption (Silva et al. 2015) and both osteoclast and osteoblast activity is influenced by PTH (Barrett et al. 1997). Further investigation is needed to clarify the stimulus of different types, intensities and duration of exercise on PTH concentrations and ultimately how this relates to bone health over time (Wherry et al. 2021b, Dolan et al. 2022)

Activities involving exercise of higher intensity and duration are similarly most likely to elicit bone remodelling. During endurance exercise, iCa is known to drop markedly in the first 20 minutes of exercise (Bouassida et al. 2003) and is associated with an increase in PTH concentrations. Indeed, a study of runners which monitored blood markers during and for 4 days post 60 minutes of running at 55, 65 and 75% VO_{2max} found that PTH and β-CTX-I concentrations were increased with higher

exercise intensity. However, although P1NP was raised during exercise, this was not intensity dependent (Scott et al. 2010). A similar finding was noted for male cyclists performing exercise tests at higher and lower intensities (Maimoun et al. 2006). Very short duration (Kristoffersson et al. 1995) and low intensity activities (Morgan et al. 2015) seem less likely to cause changes in bone turnover markers.

While bone turnover markers are the best tools available, much remains to be clarified in their interpretation and best practice protocols for use in the interpretation of exercise related changes to bone metabolism (Dolan et al. 2022). There is suggestion that changes to bone turnover in response to acute exercise are dominated by bone resorption and, as such, markers of bone breakdown rather than formation may be more useful to measure (Dolan et al. 2022). Other factors requiring careful consideration include standardisation of diet, vitamin D status, time of day and prior exercise as inconsistencies may obscure findings (Bonjour et al. 2014, Dolan et al. 2022).

2.2 Bone Stress Injuries in Sport

Bone stress injuries are an overuse injury occurring as a result of excessive repetitive force to the skeleton with an imbalance in bone metabolism favouring microdamage accumulation over its removal and replacement with new bone via targeted remodelling (McBryde 1975, Hoenig et al. 2022) and, in practical terms, may occur when the stress of training is not balanced with adequate recovery (MacKnight 2017). Stress fractures have been reported to represent between 0.5 and 20% of all injuries in athletic populations (Snyder et al. 2006). They occur across a variety of endurance sports and have stimulated research interest into possible nutrition contributors to fracture risk in athletes (Myburgh et al. 1990, Bennell et al. 1998, Dubravèiæ-Šimunjak et al. 2008, Wentz et al. 2012).

Bone stress injuries are multifactorial and, not surprisingly, it can be difficult to determine causation especially given limitations in study design which are mostly cross-sectional in nature (Table 1). In a review relating to female athletes, where sex already provides a higher risk of bone stress injuries, Abbott et al (Abbott et al. 2020) identified intrinsic risk factors for bone stress including prior bone stress, menstrual dysfunction, low or high body mass index (BMI), low lean mass, high fat mass, low bone mineral density (BMD), age, low vitamin D status or calcium intake, female athlete triad, low energy availability (LEA) or weight loss. Extrinsic factors were described as biomechanical factors, a new training mode or changes to training load. Although this list was derived for females, it is likely to have crossover for males, with low testosterone (TES) being an additional intrinsic contributor (Bennell et al. 1996a). Similarly, Nattiv et al (Nattiv et al. 1997) present a model of bone stress injury

as the interplay of mechanical, hormonal, nutritional and genetic factors influencing the balance between bone modelling and remodelling.

2.3 Rib Stress Fracture

In the elderly, traumatic fractures of the rib have been associated with age, BMD, morphological factors such as rib length and cortical thickness (Liebsch et al. 2021) but research conducted in rowing populations is limited. A summary of the existing publications is provided in Table 1: Rib Stress Injury in Athletes and an overview of key studies and their findings is now presented. Vinther et al (Vinther et al. 2005), assessed 14 Danish national team members for BMD of the lumbar spine, femoral neck and distal radius. Seven cases of rib stress fracture were identified through medical imaging and matched by gender, age, height, weight, and rowing experience in the control group. While still within the normal range, rowers with a history of rib stress fracture had significantly lower lumbar spine BMD and a trend to lower femur BMD than controls. Although this is an interesting finding, the sample size was small and was split across sex and rowing category, making extrapolation difficult.

A similar study conducted in 21 female lightweight rowers (12 active, 9 retired) measured BMD in the lumbar spine, femoral neck and radius. A questionnaire was used to collect self-reported information on history of rib pain, amenorrhoea, and training history. Those athletes who reported rib pain or oligomenorrhoea/amenorrhoea had significantly lower spine Z-scores (Dimitriou et al. 2014).

Baker, Buchanan and Bemben (Baker et al. 2022) measured a range of factors including BMD (AP spine, femur, radius and rib), geometry and skeletal asymmetries as well as using questionnaires to assess calcium intake and total bone specific physical activity scores (BPAQ) in 24 female collegiate rowers and 24 controls. Calcium intake, BPAQ, symmetry index of hip strength were the best predictors of injury risk with no BMD differences seen between injured or uninjured rowers or controls. Here, the small sample size creates a risk of bias and as the subjects were exclusively open weight females, extrapolation to rowing in general may not be appropriate.

Anthropometric characteristics could potentially contribute to rib stress fracture risk with a number of studies suggesting stroke technique and differences in upper and lower body strength as contributors (Bojanic et al. 1998, Vinther et al. 2006, Verrall et al. 2014). Body proportions such as arm span, sitting height relative to stature or leg length also potentially change the way load is applied through the trunk and may influence risk. Other untested mechanisms which have been proposed to contribute to rib stress fractures are changes to bone loading that might occur through scenarios such as changes in equipment, training prescription or loading pattern (e.g., changing the

side of the boat rowed on in sweep rowing or switching between sweeping and sculling) (McDonnell et al. 2011). Finally, a follow up study by Vinther et al (Vinther et al. 2006), found altered movement patterns in those who had a history of rib stress fracture with more upper body loading through the mid-drive and stronger arms relative to legs relative to controls.

Further research is required to better understand the nutrition and anthropometric contributors to rib stress in rowers. Given the paucity of research in rib stress injuries, the subsequent section of this review will focus on factors contributing to bone stress more generally in sport.

Table 1: Rib Stress Injury in Athletes

Study	Population	Parameters	Study Design	BMD site	Findings/conclusions
Holden (Holden et al. 1985)	7 athletes (4 F elite rowers, and 1 each from tennis, golf, gymnastics)	Clinical records	Case series	Not reported	The first published cases. Suggested may be more common in women, associated with resistance training, increased training load or with changed biomechanics due to a new oar shape. Metabolic factors and the 'pull' of the diaphragm or serratus anterior on the rib cage as possible contributors.
McKenzie (McKenzie 1989)	1 M national level rower	Clinical records	Case report	Not reported	Increased training load in preparation for competition.
Christiansen (Christiansen et al. 1997)	6 national level rowers (2 F 4 M) 3 lightweight 3 heavyweight	Clinical records	Case series	Not reported	Attributed injury to increased training load and change in equipment (oars).
Bojanic (Bojanic et al. 1998)	1 M Olympic level heavyweight	Clinical records	Case report	Not reported	Attributed to technical change.
Karlson (Karlson 1998)	10 elite level rowers 3 M (lightweights) 7 F (5 heavy, 2 lightweights)	Clinical records	Case series	Not reported	Serratus anterior or abdominal muscles may cause bending of the rib and causing fracture and changes to technique were discussed as possible protective strategies. The change to the oar shape was discussed.
Galilee-Belfer (Galilee-Belfer et al. 2000)	1 F collegiate heavyweight rower	Clinical records	Case report	Not reported	Contributors may be hormonal factors in women influencing BMD, changes in training duration/intensity, muscular fatigue or micro-trauma to bone.

Study	Population	Parameters	Study Design	BMD site	Findings/conclusions
Dragoni (Dragoni et al. 2007)	9 M Olympic level Italian rowers (7 heavyweights and 2 lightweights)	Clinical records	Case series	Not reported	Concluded a multifactorial causation including sex related factors, rowing technique, type of equipment and training status.
Smoljanovic (Smoljanovic et al. 2011)	1 M Paralympic level adaptive rower, arms and shoulders class	Clinical records	Case report	Not reported	Fracture occurred after 5 weeks of increased frequency volume and intensity of training. The chest strap required may add pressure to rib cage.
Gerrie (Gerrie et al. 2016)	2 M collegiate baseball players	Clinical records	Case series	Not reported	Vitamin D status was normal. Injury was attributed to training load.
Reid (Reid et al. 1989)	40 F elite rowers, AIS scholarship holders	Clinical records	Cohort study	Not reported	7 out of 40 presented with 13 chest wall issues including 3 stress fractures.
Hickey (Hickey et al. 1997)	172 elite rowers 84 F (15 cases), 88M (2 cases)	Clinical records	Cohort study	Not reported	Greater prevalence in female athletes noted, causation not determined, reduced upper body strength or related to hypothalamic pituitary suppression suggested.
Iwamoto (Iwamoto et al. 2011)	25 rowers (23 M, 2 F)	Clinical records- sex, age, prevalence	Cohort study	Not reported	Rib stress in rowing represented 9.5% of stress fractures presenting at a sports medicine centre.
Verrrall (Verrall et al. 2014)	45 national level rowers, 12 international rowers	Clinical presentation- competition level and injury status	Cohort study	Not reported	Rib stress caused the greatest time loss to training, was more likely in international rather than national level rowers.

Study	Population	Parameters	Study Design	BMD site	Findings/conclusions
Harris (Harris et al. 2020)	151 elite rowers	Clinical presentation, prospective analysis of medical records	Cohort study	Not specified	Period prevalence 4-15.4%, incidence 0.27-0.13 per 1,000 athlete days. Stress fracture resulted in a median 69 days (56-157 days) off water, stress reaction 57 (45-78 days). LEA and BMD identified as factors.
Trease (Trease et al. 2020)	153 Australian international level rowers	Clinical presentation	Cohort study	Not reported	64 cases of chest wall injury (16% prevalence). Females 1.4 times increased relative risk.
Wajswelner (Wajswelner et al. 2000)	74 rowers (34 M, 40 F) – elite, club and school	Peak chest muscle electromyography activity and rib cage compression	Repeated measure, within groups.	Not reported	The ribs may undergo compressive stress from the obliquus externis abdominis during the rowing stroke.
	4 F (2 heavy, 2 lightweights) 10 M (all lightweights) Danish National team rowers 7 cases and 7 matched controls	BMD, medically diagnosed RSF, confirmed by imaging	Case control	Lumbar spine (L2-L4) Femoral neck (bilateral) Distal radius (dominant side)	RSF had normal but lower spine BMD than controls.
Vinther (Vinther et al. 2006)	4 F (2 heavyweights, 2 lightweights) 10 M (all lightweights)	EMG and 2-D video analysis. Measurement isokinetic muscle strength.	Case control	Not reported	Greater thoracic muscle contraction through mid-drive and greater arm strength relative to leg strength for RSF vs controls.

Study	Population	Parameters	Study Design	BMD site	Findings/conclusions
	Danish National team rowers 7 cases and 7 matched controls				
Reid (Reid et al. 2008)	22 F lightweight rowers, current and retired, elite to club level	DXA BMD, questionnaire self-report for medical, menstrual, training and injury history.	Case control	Lumbar spine Femoral neck Total body	73% menstrual dysfunction, BMD within normal range but those with menstrual dysfunction had lower lumbar spine and total body BMD. Those with reported history of chest wall injury had lower total body BMD than those without.
Dimitriou (Dimitriou et al. 2014)	29 F club level lightweight rowers (12 active, 9 retired)	BMD, self-report questionnaire “rib pain”	Case control	Lumbar spine (L2-4) Femoral neck Total body	Lumbar spine Z scores were lower in those reporting menstrual dysfunction or rib pain.
Baker (Baker et al. 2022)	24 F collegiate rowers 24 F controls	BMD, geometry, skeletal asymmetry, calcium intake, BPAQ, symmetry index of hip strength index	Case control	Lumbar (L1-4) Proximal Femur (femoral neck, total hip, trochanter)	Predictive regression models developed comprised calcium intake and BPAQ. Calcium intake was lower in those with injury history.

2.4 Factors Contributing to Bone Stress Injury in Sport

2.4.1 Bone Mineral Density

Although physical activity is generally considered to improve bone health (Kohrt et al. 2004), there is some evidence that athletes may not always receive the expected benefits. For example, Amorim et al (Amorim et al. 2021) found both male and female dancers had consistently lower BMD than controls over a three-year study period. Meanwhile, both female (Sherk et al. 2014) and male (Barry et al. 2011) cyclists have shown a reduction in BMD over a season. Similarly, training for both triathlon (McClanahan et al. 2002) and swimming (Gomez-Bruton et al. 2017) was not seen to benefit BMD over the study period of 6 and 8 months respectively.

BMD in rowers and the response to training is relatively undescribed focussing mostly on non-elite cohorts. Masters rowers (14 M) compared with less experienced rowers and controls were seen to have higher total and regional BMD than age matched controls (Sliwicka et al. 2015) a finding not replicated in young rowers (Cohen et al. 1995, Lariviere et al. 2003). Elite male rowers were found to have higher BMD than other athletic populations such as triathletes or swimmers (Jurimae et al. 2006).

In attempting to assess the impact of rowing training on BMD, 16 experienced and 19 novice rowers were monitored before and after following the same 6-month training programme with the experienced rowers showing an increase in spine BMD whilst the novice rowers did not. The authors propose that the higher force on the blade achieved by the experienced rowers increased bone loading and, therefore, the stimulus to remodel (Lariviere et al. 2003). In contrast 17, male novice rowers monitored over a seven-month training block showed no change in hip but a significant increase in spine BMD (Cohen et al. 1995). Young et al found no change in spine BMD over a 9-month training block in 11 collegiate rowers (6 M, 5 F) (Young et al. 2014). Meanwhile a study of elite rowers over a 10-month period reported no change in BMC or BMD in females (Kurgan et al. 2018) and only an increase in arm BMD in males (Jurimae et al. 2006). The studies share a small sample size and short monitoring periods, with participants from a variety of ages, sex and training status categories making it hard to draw any conclusions as to the impact of rowing on BMD.

Sex differences may also be important. Distance runners monitored for changes in BMD over a training season showed sex differences with males tending to remain stable or increase BMD and females tending to have reductions in BMD over time despite similar reported EA (Infantino et al. 2021). In an elite rowing cohort (n= 125, 72 M, 53 F) point in time BMD was above population norms (Z score) for males and females alike with few cases of osteopenia for either AP spine (5.6%) or proximal femur (1.6%) and none with osteoporosis. Lightweight female athletes had lower BMD Z score than open weight females (Lundy et al. 2015). In those with serial measures (n= 20, 13M, 7F)

over a four-year Olympiad, Z score change showed greater bone loss in males than females (-0.6 vs -0.3 per cycle AP Spine Z score, -0.4 vs -0.1 proximal femur Z score). Almost all (92%) of males and most (71%) females had negative changes for both spine and femur measures. The causes of this decline and the sex differences are unclear (Lundy unpublished data, 2016).

While the bone health of the general populations is well represented by AP lumbar spine and femur BMD these may not be the sites most associated with rib injury in elite rowers. There is a case for a wider and more specific investigation of BMD at different body sites where bone stress is most likely. Sport specific patterns have been identified in runners showing a higher BMD in the lower body relative to upper body (Nevill et al. 2003). Fredericson et al found that runners had high BMD at all bone sites loaded through running but lower BMD at non loaded sites whereas soccer players had higher BMD than controls at all sites (Fredericson et al. 2007). Lumbar stress fractures are common in cricket fast bowlers and custom analysis of the DXA BMD scan including separation of dominant and non-dominant sides for bowling, showed higher BMD on the loaded side of the vertebrae than the unloaded side (Alway et al. 2019).

In the case of rib stress injury in rowers, there is little published data regarding rib BMD or its potential link to rib stress fracture. Smith et al, (1993) reported Rib BMD was higher in male rowing participants (n=12, training status not described) than triathletes or sedentary controls (Smith et al. 1993). Baker et al (2020) found that in 24 collegiate female rowers rib BMD was not different to sedentary controls or between those with and without rib stress injury (Baker et al. 2022). It is possible that increases in rib BMD occur alongside training history and are protective of rib stress injury. Additionally, these may not be identifiable at a lower level of training specialisation and the contributors for injury could be different between elite and sub-elite athletes. Given the evidence for localised changes to BMD at sport specific sites and the link between BMD and bone stress injuries, interest in rib BMD and associate with rib stress injury is justified.

2.4.2 Calcium, Vitamin D and Vitamin K

Calcium, vitamin D and vitamin K are micronutrients where suboptimal status has been associated with both BMD and stress fractures. Calcium is a major structural component of bone and its intake is related to the development of peak bone mass in adolescence (Johnston et al. 1993). Typical calcium intakes of elite level rowers have been infrequently described but appear relatively high in young female rowers (1187-1277mg) compared to the age-matched females from the general population (816-826mg) (Lariviere et al. 2003, Baker et al. 2022). Vitamin D is a fat-soluble vitamin found in small amounts in the diet (ergocalciferol) with the predominant source being the action of sunlight on skin (cholecalciferol). Low vitamin D status (25-hydroxyvitamin D) reduces calcium and phosphate absorption from the intestine and stimulates PTH production as a result (Kuchuk et al.

2009). Unpublished data on Vitamin D status, collected during the provision of nutrition services in a subset of the 2011 Australian rowing shadow squad, showed that 24% (n=4) had frank deficiency (<50 nmol/L) and a further 35% (n=6) had levels consistent with insufficiency (<75 nmol/L) (unpublished, Lundy, 2011). Vitamin K is a fat-soluble vitamin responsible for the chemical modification of calcium-binding proteins involved in both blood coagulation and bone such as osteocalcin. Vitamin K1 (phylloquinone) is found in green leafy plants and can be converted to vitamin K2 (menaquinones) by bacteria and found in cheese and fermented soy products (Australian National Health and Medical Research Council et al. 2006). The recommended dietary intakes for vitamin K are based on requirements for normal blood clotting and may not be sufficient for optimisation of bone health (Sokoll et al. 1997). Vitamin K status in rowing populations is currently unknown. The potential influence of these nutrients on bone stress injuries is considered below.

A study in elite figure skaters (n=412, 245 females, 167 males) assessed dietary calcium intake (consumption of dairy products) within a retrospective questionnaire covering a range of potential contributors to stress fracture in both junior and senior skaters (Dubravèiæ-Šimunjak et al. 2008). The regularity and frequency of meal consumption, eating disorders, use of food supplements and consumption of dairy products were not found to be related to stress fracture history. Similarly, no relationship was found with menstrual history.

Wentz et al (Wentz et al. 2012) assessed dietary and training predictors of stress fracture in 59 female runners (27 cases and 32 controls). BMD was measured, while information was collected on menstrual status, diet and dairy intake and training history. Taken individually, no difference was found between controls and cases for BMD at the sites measured, training factors, prior or current menstrual history, use of oral contraceptives, serum oestradiol levels or current or historical dairy intake. However, a logistic regression model of factors associated with fractures indicated current dietary calcium, irregularity of menstrual cycle, length of time running, total body BMD and running surface were the most important factors in developing a fracture.

Similarly, in a population of sports clinic patients (Myburgh et al. 1990), 25 female athletes with stress fractures were found to have lower BMD, menstrual irregularity, and lower calcium intakes than their matched controls. Nevertheless, a study by Bennell et al (Bennell et al. 1998) in male and female track and field athletes found female athlete with stress fracture had lower total body bone mineral content, lower spine and foot BMD, later menarche, fewer menses per year, less lower limb muscle mass and a lower fat intake than their controls. Here, calcium intake, diet restriction, height, weight or body fat levels were not different between cases and controls. For male athletes there was no significant difference between the cases and controls for any variable measured.

Army recruits represent a population with a relatively high rate of lower limb stress fracture and may offer clues as to contributors to stress fracture in athletes. In a study by Cline et al, (Cline et al. 1998) 127 female soldiers (49 cases and 78 controls) completed a DXA BMD assessment along with a retrospective questionnaire assessing calcium intake among other factors. Neither BMD nor calcium intake was associate with stress fracture history. In contrast, Lauder et al (Lauder et al. 2000) found a strong negative association between femoral neck BMD and stress fracture risk in another group of female soldiers. Those with stress fractures were also likely to be newer recruits and to complete more training than their counterparts who did not experience such fractures. Male military recruits followed a similar pattern with those experiencing stress fracture having a lower BMD than the control group for some types of stress fracture (femoral and calcaneal) but not others (diaphysis, tibia, fibula, metatarsus) (Pouilles et al. 1989).

Nutritional risk factors were identified in a study of new military recruits, with those who suffered lower limb stress fractures found to have lower baseline intake of calcium and vitamin D (Moran et al. 2012). This finding was replicated in a study of Finnish military recruits (Ruohola et al. 2006) which found that those with stress fractures had lower vitamin D status than those without. Further, a large scale, double-blind, randomised control trial in 3,700 female navy recruits found that supplementation with vitamin D (800 IU) and calcium (2,000mg) during 8 weeks of basic training, reduced stress fracture incidence by 21% (Lappe et al. 2008). This is an interesting finding as supplementation could not be expected to influence BMD over the short study period and suggests supplementation may have reduced microdamage through another mechanism. Amenorrhoea during basic training was also identified as a significant risk factor for developing stress fracture.

The effect of vitamin K and bone health has been assessed in two meta-analyses in the general population which indicate potential benefits to supplementation (Cockayne et al. 2006, Fang et al. 2012). To date, however, only two small studies have been conducted in female athletic populations with inconclusive findings. Braam et al (Braam et al. 2003) measured BMD in female endurance athletes after 2 years of vitamin K supplementation or placebo and found no difference between groups. In contrast, Craciun et al (Craciun et al. 1998) found supplementation with vitamin K caused a positive shift in bone turnover markers, though this may be due to the poor baseline status in more than half the participants. Further research is needed to understand whether vitamin K has a role in BMD and bone stress injuries in athletes.

BTM also give clues as to the contribution of these nutrients to bone health. Young women given a calcium, vitamin D and K supplement over a 6-month period showed reduced CTX and increased P1NP and improvements in the cOC/ucOC ratio (Umarji et al. 2021) indicative of a positive bone remodelling. Sadideen (2004) (Sadideen et al. 2004) supplemented 17 female and 15 male subjects

with a 400mg oral calcium dose at night, followed by an overnight fast in a crossover counterbalanced intervention. Pre and post measures of BTM showed and increased iCa and urinary calcium/Creatinine and a decrease in both PTH and β-CTX-I with supplementation. The authors concluded that 400mg overnight calcium was sufficient to inhibit bone resorption in young healthy adults. These findings are similar using food rather than supplements. Untrained males randomised to Greek yoghurt or placebo during a 12-week training programme showed P1NP increased more over time in the Greek yoghurt supplemented group suggesting a shift in bone turnover towards formation (Bridge et al. 2020). Similarly, five weeks of ~60g vitamin K2 rich cheese increased OC and improved cOC/ucOC ratio (Lundberg et al. 2020). Whilst it appears bone resorption may be reduced through nutrition however it is unknown whether this inhibition is ultimately beneficial in situations where microdamage is not in excess of capacity for repair and such as in young populations where bone is otherwise healthy.

2.4.3 Diet Restriction and Menstrual Dysfunction

Diet restriction and menstrual dysfunction may be a major contributor to reduced BMD and bone stress injuries in athletes. These will be discussed in the context of LEA in the following section.

2.5 Energy Availability

Energy availability is a concept that initially developed from the observation that female athletes often experienced a cluster of symptoms included hormonal changes and menstrual dysfunction (Loucks et al. 1989) and reduced BMD (McLean et al. 2001); a syndrome described as the Female Athlete Triad (FAT). Although it was initially postulated that women could not tolerate the stress of intense training or needed to maintain a high level of body fat in order to have normal reproductive function, these theories have since been disproved (Loucks et al. 1984, Loucks 2003). It was also assumed that weight stability in an athlete indicated energy balance, with sufficient energy intake (EI) to support health and exercise. However, in a series of studies, Loucks et al (Loucks et al. 1993, Loucks et al. 1994, Loucks et al. 1998b, Loucks et al. 2003b) identified that inadequate energy consumption in females could cause suppression of the hypothalamic pituitary axis, 'saving' energy on bone repair and maintenance and reproductive function to return to energy balance. While such an adaptation defends the loss of body mass, it may have other deleterious outcomes on health. It is thought that insufficient EI triggers a reduction in gonadatrophin releasing hormone (GnRH), interruption to luteinizing hormone (LH) pulsatility and downstream changes to the endocrine system (Figure 4). This system of energy conservation is logical from an evolutionary perspective, delaying costly energetic processes such as reproduction in times when food is scarce and reinstating when more plentiful. Similarly, the repair and maintenance of bone may be less immediately

pressing for survival than providing adequate energy to run essential organs (Fiskerstrand et al. 2004, Oliveira-Junior et al. 2022, Shirley et al. 2022)

The term Energy Availability helps to describe the requirement for energy for both exercise and bodily functions. It is defined as the ingested energy (EI) remaining for all other metabolic processes after the energy cost of exercise (EEE) has been subtracted and is expressed relative to fat-free mass (FFM) to represent the body's most metabolically active tissues (Loucks et al. 2003a). LEA may occur accidentally through a misunderstanding of the energy needs for sport or because of dietary restraint, disordered eating or eating disorder (Mountjoy et al. 2014). Methodological consistency is lacking in LEA measurement which makes assessment of prevalence difficult, but risk is thought to be higher in leanness or aesthetic sports and those with higher training loads (Mountjoy et al. 2018).

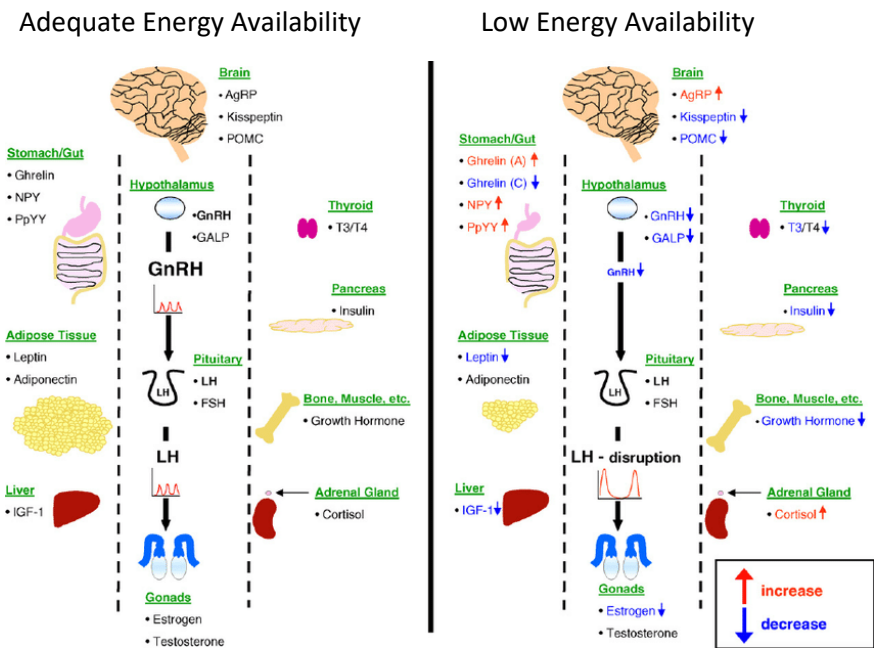


Figure 4 The impact of low energy availability on the hypothalamic pituitary axis (Martin et al. 2008)

Continued evolution of the awareness and understanding of LEA has identified that its effects extend beyond menstrual function and bone health (Mountjoy et al. 2014), and manifest in male as well as female athletes. This growing awareness gave rise to the concept of Relative Energy Deficiency in Sport (RED-S) (Mountjoy et al. 2014), which provides broader understanding of the potential effect of LEA on multiple body systems across both sexes. These may include gastrointestinal symptoms, dyslipidaemia, hypotension, impaired immune function, hypothyroidism, and negative psychological impacts (Figure 5). LEA is also thought to increase risk of injury, both bone (Barrack et al. 2014, Tenforde et al. 2021) and soft tissue (Rauh et al. 2010) as well as to impair body composition management and sports performance through impaired adaptation to training (Vanheest et al. 2014, Murphy et al. 2022). Whilst further research is needed, the effects of LEA are evident on both

endurance (Vanheest et al. 2014, Ackerman et al. 2019) and neuromuscular performance (Tornberg et al. 2017).

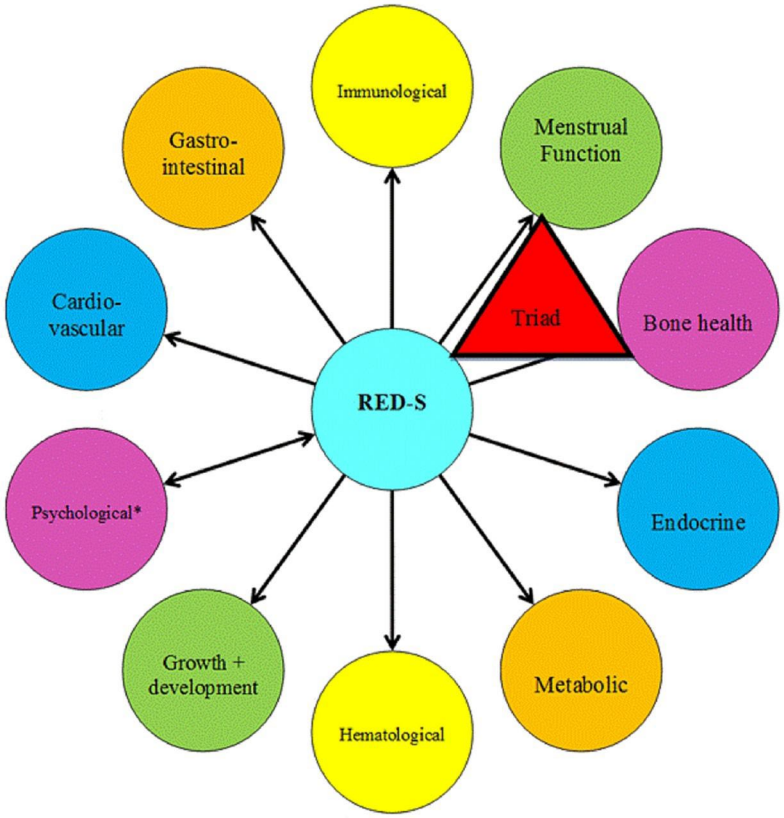


Figure 5 Relative Energy Deficiency in Sport (Mountjoy et al. 2014)

2.5.1 Energy Availability in Male Athletes

The IOC consensus paper on RED-S includes male athletes as a population of concern (Mountjoy et al. 2018). There is increasing evidence that male athletes suffer from the outcomes of LEA with evidence of disordered eating behaviour (Filaire et al. 2007, Bratland-Sanda et al. 2013, Goltz et al. 2013), altered hormone concentrations (Ayers et al. 1985, MacConnie et al. 1986, Hackney et al. 1988, 1990, Degoutte et al. 2006, Hagmar et al. 2013), changes to immune function (Hagmar et al. 2013, Abedelmalek et al. 2015) impaired bone health (Olmedillas et al. 2011, Dolan et al. 2012, Guillaume et al. 2012) and reproductive function (Cumming et al. 1989).

Endurance trained men have long been recognised as experiencing changes in their hypothalamic-pituitary-gonadal (HPG) axis, with low TES and hypogonadism being studied under the umbrella of the exercise hypogonadal male condition (reviewed in (Hackney 2020)). The stress of training is proposed as the primary driver of this model and LEA being considered a separate issue. Whether these are two separate conditions or chronic and acute presentations of the same pathology is still

under debate (Hackney 2020). Male endurance runners with EHMC also shower lower EA and higher rates of osteopenia than those without EHMC (Hooper et al. 2017). Further, a case series examination of young men with low TES and hypogonadotropic hypogonadism highlights that increased EI was associated with improved TES levels and, although training load was poorly described, analogous to change seen with the Female Athlete Triad and RED-S (Wong et al. 2019). Recently the Female Athlete Triad Coalition has proposed a Male Athlete Triad while recognising gaps in the research knowledge for prevalence, identification, and management (Fredericson et al. 2021, Nattiv et al. 2021).

There have been relatively few studies specifically investigating LEA in male athletes with most of the available research focussing on situations of energy restriction. These studies can provide clues as to how LEA may present itself in active males (Table 2). Several of these are in military populations and have the advantage of large sample size, although because the energy restriction is often severe and combined with other stressors such as sleep deprivation, direct comparison with athletes is more difficult (Friedl et al. 2000, Alemany et al. 2008). Collectively these studies suggest energy restriction in active men may result in reductions in total and free TES, IGF-1, thyroid hormones, and an increase in cortisol.

Early studies in male athletes including runners, triathletes (Fudge et al. 2007, Drenowatz, 2012 #560) and professional cyclists (Vogt et al. 2005) identified that inadequate EI relative to training load (without directly quantifying EA) was associated with reduced BMD. Others have used proxies (discussed in more detail in section 2.6 Tools for Identifying Low Energy Availability) such as resting metabolic rate suppression, exercise dependence or disordered eating screening tools. For example, Sesbrano et al (2021) found elite volleyball players with higher scores for emotional eating using the Three Factor Eating Questionnaire also scored higher for patellar injury (Sesbreno et al. 2021). Studies collecting dietary data in athletes have noted estimated EI below that of exercise energy expenditure (EEE) and attributed this to underreporting which, while possible, neglects consideration of LEA as an alternative cause (Brinkmans et al. 2019). Conversely, the likelihood of miscalculation or errors of reporting for both EI and EEE makes detecting LEA in these populations equally open to error (Burke et al. 2018b).

Studies specifically describing LEA in males are summarised in Table 3 and suggest divergent estimates of between 0-83% with higher rates in weight sensitive sports (Koehler et al. 2013) and with increasing EEE (Koehler et al. 2013, Jurov et al. 2021). Using a uniform threshold for LEA for men as for women there appears to be a similar occurrence for both sexes (Heikura et al. 2018b, Beermann et al. 2020) and a higher rate in athletes as opposed to controls (McCormack et al. 2019). There is a lack of consistency in the methodology for calculation of both EI and EEE which likely contributes to the wide variance in estimates. For EI these differences include but are not limited to

the use of food diaries or food frequency questionnaires and the number of days assessed, time of the season and whether a non-training day is included as part of the analysis. For EEE the tools used to assess EEE can vary widely in their estimates. These include derivation from heart rate data, actigraphy, physical activity logs using estimates from the compendium of activity or global positioning system data. These limitations are outlined in detail in the review by Burke et al (Burke et al. 2018b). There may also be a bias towards assessing athletes more likely to be at risk of LEA, such as those from leanness sports (Gibbs et al. 2013). Indeed, current reproductive function, as assessed by questionnaire and blood markers, may be more accurate in identifying those with LEA than an EA assessment itself (Heikura et al. 2018a).

Controlled studies of LEA in men are few and show contrasting results within this literature and in comparison, to studies of women. Koehler et al (Koehler et al. 2016), investigated LEA in six active young men using four interventions in a repeated measures cross-over design. Two interventions induced LEA of 15 $kcal.kg^{-1}$ $FFM.day^{-1}$, either through diet restriction alone, or through a combination of diet and exercise. Similarly, the adequate energy availability conditions provided 40 $kcal.kg^{-1}$ $FFM.day^{-1}$, one with diet alone and the other with an increased diet intake to compensate for an exercise prescription. These interventions were completed for four days with a four-day washout in between, while changes in body mass and fasting concentrations of various hormones were assessed. Leptin, insulin, weight and fat mass were reduced while TES, ghrelin, IGF-1 and fat free mass were unchanged by the intervention. This finding is in contrast with several other studies which have shown a reduction in total TES in response to energy restriction (Hackney et al. 1988, Bilanin et al. 1989, Hackney et al. 1990, Degoutte et al. 2006, Dolan et al. 2011, Hagmar et al. 2013). The authors concluded that LEA was a significant stressor but there may be a higher threshold for disruption to the hypothalamic pituitary axis in men than in women.

Methodological differences are noted between these studies of LEA in free-living males and the defining studies on energy availability in females (Loucks et al. 1993, Loucks et al. 1994, Loucks et al. 1998a, Loucks 2003). Indeed, in these early investigations, EI was tightly controlled by the provision of formulated diets and hormonal measures were taken at regular intervals over a 24-hour period. Although the more recent studies on male athletes have focussed on changes to fasting hormone concentrations, it is possible that changes in pulsatility or pulse amplitude of LH which were not assessed may have occurred as an early marker of LEA. Indeed, a study examining these characteristics in men following a 48 hour fast found decreased FSH, LH and LH pulsatility alongside change in TES (Cameron et al. 1991). Replicating these measures at increments of EA seems important to furthering our understanding of LEA in males. The impact of LEA induced by diet alone or a combination of diet and exercise may also have different outcomes. A secondary analysis of the fasting study showed that LEA with exercise was protective of subjective indices of mood, sense of

fitness, physical fitness and recovery compared with LEA due to diet restriction alone (Martin et al. 2021).

Nevertheless, support for sex-based differences in the outcomes of LEA is provided by an investigation into changes to BTM with induced LEA. Eleven men and women completed two 5-day blocks of either control (45 kcal.kg^{-1} FFM.day^{-1}) or LEA (15 kcal.kg^{-1} FFM.day^{-1}) induced by a combination of diet and exercise. While women were found to incur an increase in β-CTX-I and reductions in P1NP, insulin and leptin in response to LEA, the trends were similar but not statistically significant differences in men with a greater variance in response (Papageorgiou et al. 2017). In contrast, another study induced LEA in men (n=7) over 5 days showed reduced BTM and a shift in bone turnover to resorption (Murphy et al. 2021). However, LEA in male endurance athletes was not associated with low BMD when a threshold of 30 kcal.kg^{-1} FFM.day^{-1} was used (Lane et al. 2021). Furthermore, a study of male and female dancers reported lower BMD than controls at all sites, but this was only associated with LEA in females (Amorim et al. 2021). Similarly, BMD was lower and rates of bone injury higher in male endurance athletes with lower TES, but was not different between those with low or normal EA (Heikura et al. 2018a).

LEA was inferred, but not prescribed or measured, in 22 well trained cyclists undergoing a 4-week intensified training block. EI did not increase despite clear increases in EEE. RMR and T3 were lower and cortisol higher after the intervention. Free and total TES were unchanged (Stenqvist et al. 2020). Meanwhile, a study of 28 male elite race walkers showed no difference in IL-6, hepcidin, white blood cell counts or cortisol after 6 days of adequate EA or LEA (McKay et al. 2021) whereas 3 days of LEA resulted in raised hepcidin in male long-distance runners (Ishibashi et al. 2020). Finally, a cross sectional study of male endurance athletes was unable to show differences in performance, bloods or psychological characteristics when using a threshold of 30 kcal.kg^{-1} FFM.day^{-1} to define LEA, but differences in cognitive restraint were seen between athletes with lower resting energy expenditure (Jurov et al. 2021),

The paucity of this literature and diversity in methodology and findings raise the question of how LEA operates in men, whether cut points derived in women can be used in men and more broadly the utility of the calculation of EA itself. It is possible that men need either a greater magnitude or duration of restriction or both in order for perturbations in body systems to be apparent but further research is required.

2.5.2 Low Energy Availability, Bone Metabolism and Risk of Stress Fracture

Diet restriction and LEA have been identified as contributors to reduced bone health and menstrual dysfunction, which independently and collectively pose potential risk factors for stress fractures in

athletes (Zanker et al. 2004, De Souza et al. 2008, Gibbs et al. 2014). Evidence of the direct effect of LEA on bone metabolism was demonstrated in an early study in which 29 eumenorrheic young women were exposed to five days of LEA (10, 20, 30 kcal.kg^{-1} LBM.day^{-1}) or adequate EA (45 kcal.kg^{-1} LBM.day^{-1}) (Ihle et al. 2004). Although markers of bone breakdown and formation were both affected by the stepwise restriction of EA, there were differences in the pattern of response. For example, urinary NTX (a marker of bone breakdown) was affected at a threshold of 10 kcal.kg^{-1} LBM.day^{-1}, whereas PICP (bone formation) had a linear relationship with EA, with its decrease aligning to the magnitude of the EA restriction. Meanwhile OC was reduced at all EA targets from 30 down to 10 (Ihle et al. 2004). A similar picture comes from a study in 8 male distance runners who were prescribed either 50% or 100% of their energy requirements for 3 days in conjunction with training. P1NP and IGF-1 both declined in response to low intake, but NTX was unchanged. A cross-sectional study of male distance runners with LEA (18.9 ± 6.8 kcal.kg^{-1} FFM.day^{-1}) reported urinary NTX markers above the reference range (Taguchi et al. 2020).In rowing more specifically, adolescent females with menstrual dysfunction were shown to have a lower osteogenic response to rowing training than those with a normal menstrual cycle (Morris et al. 1999) and male lightweight rowers undergoing an acute fast (24 hours) had lower levels of both OC and urinary pyridinium crosslinks suggesting reduced bone turnover during fasting (Talbott et al. 1998).

Whilst these are acute responses, if repeated over time they could significantly and negatively impair on bone health. Indeed, the foundation of the female (De Souza et al. 2017) and male (Nattiv et al. 2021) athlete triad is the observation of the co-existence of LEA, reproductive dysfunction and poor bone health and injury. There are numerous examples of such clusters, including cohorts of female athletes with stress fracture were found to be more likely to have menstrual dysfunction, stress fracture history, high training load, lower OC and ucOC than those without (Miyamoto et al. 2018). Similarly, male distance athletes had a 4.5 times increased incidence of bone stress when they had TES in the lowest quartile of the reference range and female distance athletes showed an association of the female athlete triad cumulative risk assessment score or REDS- CAT score with bone injury (Heikura et al. 2018a).

Stress fractures in athletic populations are discussed more broadly in section 2.2 Bone Stress Injuries in Sport but it appears clear that energy availability needs to be considered when nutrition contributors to bone stress injury are investigated.

Table 2: Energy Restriction in Active Men

Study	Population	Design	Markers	Results	Conclusions
Anorexia Nervosa & Fasting					
Cameron (Cameron et al. 1991)	8 healthy men	48 hours of induced fasting	Samples collected at 15 min intervals between 0800 -1600 LH, FSH, TES pulse frequency and amplitude Cortisol hourly mean	Following fasting ↓ mean plasma LH and ↓ pulsatility ** ↔ LH pulse amplitude ↓ mean TES* and FSH** ↔ cortisol	Acute undernutrition slows the central drive to the reproductive axis
Sabel (Sabel et al. 2014)	14 M anorexia nervosa (AN) patients, admitted to an eating disorder (ED) unit)	Case series	REE BMD Blood markers	10% of ED admissions were males with severe AN TES was 'markedly low' RMR ratio = 0.78 (suppressed) BMD low in 11 of 14 patients TSH normal	Severe undernutrition in males presents with low TES, reduced BMD and REE suppression.
Silla (Silla et al. 2021)	53 M 36 AN, 17 controls	Retrospective chart review.	Height, weight, medical history, blood parameters- FBC, electrolytes, hormones, injury.	AN had ↓ TES, ↑incidence of traumatic fracture, 38% had cardiovascular complications, Gastrointestinal problems were less common than in F	M may present with milder undernutrition than F with AN. Overexercising is most likely to be the primary weight control method.

Study	Population	Design	Markers	Results	Conclusions
Military					
Friedl (Friedl et al. 2000)	97 healthy young males in US Army Rangers course	4 phases of energy restriction and refeeding, 2 weeks each. Also subjected to sleep restriction and thermal stress (daytime heat and humidity, no shelter at night). Group 1 (n=49) average energy deficit of 1200 Cal/day Group 2 (n = 48) average energy deficit of 1000 Cal/day	Collected fasted (0500 - 0800) • Skinfolds, abdominal girth, DXA body composition • Insulin, BGL • GH, IGF-1 • Thyroid hormones (TSH, T3, T4) • Sex hormones: LH, SHBG, Total and free TES • Serum lipids (total, LDL &, HDL cholesterol, TG)	Insulin and BGL ↓ throughout* TG ↓*, HDL ↑ progressively *LDL ↓ to W2, then progressively ↑* TSH ↑ with restriction, ↓ in refeeding*, T3, T4, IGF-1 ↓ W4 then plateaued, quick to respond to refeeding and sensitive to degree of energy restriction (↓↓ group 1, ↓ group 2)* Cortisol ↑throughout, faster in Group 1 than 2* TES ↓↓ well below the normal range and responded quickly to refeeding* LH ↓ and returned to baseline with refeeding*	• ↑ weight loss = ↓ final serum IGF-1 and ↑ HDL. • ↓ body fat reserves ↑ total cholesterol and ↓ T3. • ↓ TES with ↑ proportion of weight loss contributed by fat • Responses were graded to the severity of energy deficit for T3 and IGF-1 • Threshold effect of energy deficit for TES change • Cholesterol and cortisol appear to respond to prolonged energy restriction • TES, T3 and IGF-1 respond more acutely to energy restriction.

Study	Population	Design	Markers	Results	Conclusions
Alemany (Alemany et al. 2008)	34 M Military	Mixed model, repeated measures, randomly assigned, blinded G1 0.9g/kg protein G2 0.5g/kg protein Both completed 8 days energy deficit High EEE 16.5 MJ/day Low EI 6.5 MJ/day	DXA body composition Total and free IGF-1 IGFBP-1 -2- 3 Total and free TES, SHBG DHEA, DHEA-S EEE and sleep Actigraph EE DLW	Between groups ↔ weight, FFM, FM lost, EE IGF-1 ↓(more in G2)*** Total and free TES ↓ *** SHBG (more in G2)*** DHEA and androstenedione ↓**	Low energy, adequate protein diet → attenuation in decline of IGF-1 and increase in SHBG. IGF-1 and androgenic systems were altered independent of protein group. Protein intake had no impact on preservation of FFM.
Athletes					
Ayers (Ayers et al. 1985)	20 M marathon runners (>48 km / week) 10 controls	Cross-sectional study comparing features of endurance runners and controls	Hormones- Total and free TES, oestradiol, luteinizing hormone, DHEA-S semen analysis running mileage, body fat % through skinfolds	Compared to controls runners ↓ total TES (only 6 WNL)* ↔ free T (13 well below NL) ↔ oestradiol (15 lower) ↔ sperm count	Vigorous training significantly reduces TES but not sperm production in most athletes. A subset of the running group (10%) had lower body fat, hormone levels and sperm count possibly indicating LEA.
Hackney (Hackney et al. 1988)	11 M Endurance trained 11 M untrained	Cross-sectional, compare endurance athletes and controls	Height, weight, skinfolds Fasted, rested blood samples every 15 minutes for 4 hours for LH, prolactin, cortisol, TES, TG	Endurance trained ↓ TES (total and free)** ↑LH (ns) ↔ oestradiol, LH pulsatility, LH pulse amplitude, prolactin, cortisol, TG	Endurance runners have altered reproductive hormone profiles.

Study	Population	Design	Markers	Results	Conclusions
Bilanin (Bilanin et al. 1989)	13 M Distance runners (>64km/week), 11 controls	Cross-sectional, descriptive	BMD: DXA Lumbar, radius and tibia Body composition: underwater weighing EI: 7day diet records	Compared to controls runners ↓ weight and %body fat* ↑VO_{2max}* ↓ lumbar BMD* ↔ radial and tibial BMD EI met predicted needs calcium intake >800mg/day	Propose hormonal changes associated with distance running as the cause for lower BMD in lumbar spine
Hackney (Hackney et al. 1990)	5 M Endurance trained runners 5 M controls	Reproductive hormones measured at 15-minute intervals over 4 hours for a resting condition and with administration of synthetic GnRH	TES, free TES, & free Oestradiol LH- total, pulse frequency, pulse amplitude Prolactin Cortisol	Runners ↓ TES* and TES relative to LH levels in resting condition* An attenuated LH response following GNRH stimulation* ↔ cortisol	Suggests disruptions to the HTPA, similar to those in trained females.
Vogt (Vogt et al. 2005)	11 M professional cyclists	Cross-sectional, estimation of energy cost and intake during training camp	EI 6 day weighed food records EEE SRM system BMR estimated HB equation	Weight loss 730g EI 13.5 MJ EEE 11.5 MJ Total EE 19.5 MJ	Whilst EA was not calculated it is clear there is a significant shortfall in EI relative to training load.
Degoutte (Degoutte et al. 2006)	20 M national level Judokas	Randomly assigned to weight loss (5% of body weight through own methods) or weight maintenance group.	Body composition: Weight, sum 4 skinfolds EI: 7-day food diaries Blood: TG, FFA, BGL, glycerol, ammonia, uric acid, urea, creatinine, Insulin, ACTH, cortisol, TES, thyroid hormones	In weight loss group Weight ↓** Energy restriction (4 MJ/day) Hand grip strength and isometric rowing ↓** POMS- ↓ vigour, ↑fatigue & tension*	Performance and mood adversely affected by energy restriction. Hormonal changes were induced after energy restriction for 7 days.

Study	Population	Design	Markers	Results	Conclusions
			Other: POMS inventory hand grip strength, isometric row	Hormonal- ↓TES*, T/C ratio**, T3/4 ratio* ACTH, cortisol, DHEA-S ↑** Other- TG↓, FFA, Glycerol, urea, uric acid ↑	
Fudge (Fudge et al. 2006)	9 M elite Kenyan distance runners	Cross-sectional, energy balance in heavy training block, altitude	EE: doubly labelled water EI: 7 d weighed food record	EE = 14 611 ± 1043 kJ/day EI = 13 24 ± 1330 kJ/day	Endurance athletes in a heavy training block may not meet energy needs.
Lombardi (Lombardi et al. 2012)	9 professional cyclists competing in Giro d'Italia	Cross sectional- 22-day professional cycling race.	Bone and energy metabolism markers (BAP, TRAP5b, total and undercarboxylated osteocalcin, leptin, adiponectin) hormones (cortisol, TES)	Body weight ↓ * BAP ↔, TRAP5b ↑ ** BAP/TRAP5b ↓↓*** Total osteocalcin ↓** Undercarboxylated OC ↔ Adiponectin ↑throughout ** Leptin ↓ * TES ↔, Cortisol ↓**	BAP/TRAP5b ratio indicates an imbalance towards bone resorption in prolonged exercise. Energy deficit assumed by weight loss and high EEE but EI but not measured.
Drenowatz (Drenowatz et al. 2012)	15 M endurance athletes	2 non-consecutive weeks- high and low training volume, crossover	Body composition- Bod Pod™ RMR assessment EEE- HR monitor, regression NEAT- Sensewear™ EI- Block FFQ	TDEE (RMR + EEE + NEAT) was higher than EI in both low and high training weeks EI ↔ between conditions	Underreporting was suggested as the reason for differences.

Study	Population	Design	Markers	Results	Conclusions
Dolan (Dolan et al. 2011)	20 M professional jockeys 20 M healthy age and BMI matched controls	Cross sectional study Periodic energy deficit assumed in the jockey population but not measured	BMD (DXA) – lumbar spine, femoral neck Bone turnover - NTx Hormonal profile – T4, cortisol, FSH, LH, TSH, SHBG, IGF-1, free TES, bioavailable TES	Relative to controls, jockeys ↓ BMD ** Bone resorptive activity ↑** Percent bioavailable TES ↓** SHBG ↑**	Jockeys have an elevated rate of bone loss and reduced bone mass likely associated with disrupted hormonal activity.
Hagmar (Hagmar et al. 2013)	44 M Olympic athletes, 18 leanness sports, 26 other disciplines	Cross-sectional study	Weight DXA body comp, whole body BMD (spinal section assessed) Steroid hormones POMS	Leanness sport athletes ↑ hours training** ↑ time lost to illness* ↔ injury, ↓ % body fat** ↑ spine BMD* ↓ free TES, leptin, IGFBP-1, IGF-1/IGFBP-1* ↑ POMS *	Leanness athletes demonstrate body composition and endocrine differences which may be explained by lower body fat. There was a higher rate of illness and higher global POMS score. There was no evidence of HPA suppression.
Abedelmalek (Abedelmalek et al. 2015)	11 M Judokas	Randomised cross-over design, normal diet and 7 days caloric restriction (6.7MJ/day reduction from baseline intake)	Weight, body composition (BIA), heart rate (HR), Diet intake (3-day food diaries), Specific Judo Fitness Test Blood (pre & post exercise) – leucocyte counts, lymphocytes and neutrophils, TES, cortisol, growth hormone (GH), IL-6, TNF-α	In response to caloric restriction Weight ↓* Performance on Specific Judo Fitness Test ↓* Exercise HR ↑*TES ↓, GH and cortisol ↑ * TNF-α and IL-6 ↑*	Caloric restriction reduced performance in sport specific tasks, caused hormonal perturbations and may increase the risk of illness in athletes.

Study	Population	Design	Markers	Results	Conclusions
Pardue (Pardue et al. 2017)	1 M body builder	Case study, 13 months	EA not measured but TES, fT3, T4, cortisol, leptin, ghrelin, DXA body composition and Bod Pod, RMR, Pittsburgh sleep quality index (PSQI) and actigraphy, anaerobic power (Wingate test)	During 8-mo energy restriction TES, fT3, T4 ↓, and ↑ cortisol and ghrelin RMR ↓ from 107.2% of predicted to 81.2%. Sleep and power output ↓ Changes reversed in 3-mo recovery	Energy restriction caused metabolic and endocrine perturbations in this individual and need investigation in larger samples.
Brinkmans (Brinkmans et al. 2019)	41 M Dutch Premier League Football players	Cross-sectional	EI and EEE assessed by 24-hour diet recall x 3 and DLW	Daily mean EI was 18% lower than EEE.	Underreporting was suggested as the reason for differences given there was no weight loss.
Sesbreno (Sesbreno et al. 2021)	22 M national level volleyball players	Retrospective cross-sectional	Anthropometry, DXA, RMR, Blood- Vitamin D, ferritin, B12 EI- 4-day diet records 3 factor eating questionnaire Patellar tendon injury questionnaire (VISA-P)	EI inadequate, EA not calculated. RMR was measured but not assessed for adequacy. Patellar injury rates ↑ with ↑ emotional eating score	Adequate EI was identified as important in this group but was not assessed for EA or metabolic suppression.

* P<0.05, ** P<0.01, *** P<0.001

Table 3: Summary of research relating to LEA in male athletes

Study	Population	Design	Markers	Results	Conclusions/Comments
Prevalence/Observational Studies					
Koehler (Koehler et al. 2013)	167 M, 11-25 y, variety of sports	Cross-sectional	EI, EEE, EA T3, insulin, leptin	~ 50% had EA < 30 kcal.kg^{-1} FFM.day^{-1} low or normal EA ↔ insulin, IGF-1, body fat, T3 EA was ↓ for lowest quartile leptin, in weight class sports and with ↑ EEE	It is uncertain whether self-report EA can be used to identify those with energy deficiency based on these findings. This study included children.
Viner (Viner et al. 2015)	6 M 4 F cyclists with low BMD	EA across the season – 3 time points, with comparison between male and female cyclists and contributors to LEA	EI, EEE, EA DXA BMD and body composition TFEQ	BMD ↔ across season BMD 40% low spine, 10% femur M ↑ EEE during competition compared to F EA ↔ between M and F or across the season. 70% classified restrained eaters.	There may be a high prevalence of LEA and dietary restraint in competitive, non-elite cyclists.
Hooper (Hooper et al. 2017)	9 M distance runners with EHMC 8 non-active controls	Cross-sectional, between groups comparison.	TES, LH, FSH, cortisol, BMD FFQ Aging Males Symptoms questionnaire (AMS)	EHMC groups showed ↓ TES, higher AMS, ↓ EA ↔ BMD relative to controls	EHMC have lower EA, may be a contributor to the condition. EEE measurement was not well described.

Study	Population	Design	Markers	Results	Conclusions/Comments
Silva (Silva et al. 2017)	39 M athletes 18 F volleyball, basketball, triathlon, swimming	Observational, follow up from pre-season to season end- 5-10months	DXA body comp REE TEE using DLW EI, Average EA calculated from average EI and EEE	REE ↓ with lower EA EEE ↑ over the season Triathletes had ↓ EA and greater changes in REE	Metabolic adaptation was associated with energy balance and energy availability. M & F data pooled.
Torstveit (Torstveit et al. 2018)	31 M cyclists, triathletes, distance runners club level	Cross-sectional	RMR EI, EEE, EA, EB DXA body fat Bloods- cortisol, TES, fT3, glucose	65% suppressed RMR EA ↔ low or normal RMR (37 v 41 kcal.kg^{-1} FFM.day^{-1}) ↑ cortisol, ↓ tTCR in those with the largest hourly EB deficits. Body fat ↓ in those with least time in EB deficit	Male endurance athletes with supressed RMR had similar 24-hour EB and EA but had spent more time in high energy deficit and larger single hour deficits than those with normal RMR.
Heikura (Heikura et al. 2018a)	21 M 27 W elite endurance athletes	Pre/post measures, 3-4 weeks altitude camp	Hb Mass EA, LEAF-Q Injury and Illness Q	Mean EA for men 36 kcal.kg^{-1} FFM.day^{-1}	No association between EA and HB mass change possible due to the point in time assessment.
Heikura (Heikura et al. 2018b)	24 M 35 F distance athletes	Cross-sectional	EA (low <30 kcal.kg^{-1} FFM) RED-S tool, MAT Total TES fT3 BMD	LEA 25% M, 31% F 40% in lowest quartile ref range for TES 4.5 x ↑ fracture risk for lowest quartile TES ↑ Triad CRA and RED-S CAT score correlated with ↓ T3 and ↑ bone injury	Rates of LEA symptoms were high within the group. Tools assessing physiological symptoms of LEA provide a better assessment than a snapshot EA alone.

Study	Population	Design	Markers	Results	Conclusions/Comments
McCormack (McCormack et al. 2019)	27 M collegiate cross-country runners	Cross-sectional, between groups comparisons (M v F & controls)	DXA body comp and BMD EI- Block FFQ EEE- 3-month training diary Low EA <30 kcal.kg^{-1} FFM.day^{-1} EDE-Q	Compared to controls - M runners ↑ femoral neck, total hip, total body BMD - Spine BMD ↔ - Mean EA was adequate - More M runners had LEA (42 vs 14%)	Male runners were more at risk than female runners or controls and require education. Runners in general had high scores for eating and shape concern.
Lane (Lane et al. 2019)	108 M recreationally trained	Cross-sectional, prevalence	3-day food and exercise records, questionnaire	47.2% <30 kcal.kg^{-1} FFM.day^{-11}, 33% 30-45 kcal.kg^{-1}FFM and 19.4% > 45 kcal.kg^{-1}FFM. Cyclists were more likely to have LEA than runners	Prevalence was high but further research needed to identify cut points appropriate for males. Lean mass and REE were estimated using predictive equations. Assessment of food records was poorly described.
Beermann (Beermann et al. 2020)	21 M, 20 F collegiate distance runners	Cross-sectional	EI: Block 2014 FFQ FFM: DXA EEE: Training logs RMR estimated	45% of male and 41% of female runners had EA<30 kcal.kg^{-1} FFM.day^{-1}	EA and carbohydrate intake were low in both male and female collegiate runners.
Egger (Egger et al. 2020)	8 M & 6 F wheelchair basketball athletes	Cross-sectional	Weighed 7-day food record Training diary REE EB	↑ prevalence LEA F than M 12.5% M, and 83 % F had EA < 30 kcal.kg^{-1} FFM.day^{-1}, 73% of days in LEA for F vs 30% in M. EB was positive for M and negative for F.	Females in this cohort were more at risk. Weekly mean EA values missed individuals who had days of LEA, almost all subjects M and F.

Study	Population	Design	Markers	Results	Conclusions/Comments
Lee (Lee et al. 2020)	12 M soccer players	Cross-sectional	EI: food diary, EEE: HR monitor, REE DXA: BMD & body comp POMS, EAT 26, Blood markers- β-CTX-1, FSH, LH, FT3, cortisol, bone-ALP, leptin, TES, GH	EA 31.9 kcal.kg^{-1} FFM.day^{-1}, 17%>45 kcal.kg^{-1} FFM.day^{-1} 42% LEA (< 30 kcal.kg^{-1} FFM.day^{-1}), supressed REE, ↓ IGF-1. Other hormone and bone markers ↔ by EA	LEA caused metabolic suppression but did not cause changes in bone markers or hormonal status.
Taguchi (Taguchi et al. 2020)	6 M distance runners	Cross-sectional observational	EI (3-day diary), EEE (HR, VO_2 adjusted), EA, EB, REE DXA BMD, body comp EAT 26 Blood sample: TES, fT3, IGF-1, bone-ALP, NTX, Vitamin D	83% LEA, mean 18.9 kcal.kg^{-1} FFM.day^{-1}, 67% RMR_{ratio} < 0.9 67% low Total body BMD Z score 33% subclinical low TES 100% high NTX EAT 26 scores normal range	Multiple signs of energy deficiency were seen in this group in the absence of eating disorder.
Jurov (Jurov et al. 2021)	12 M endurance trained athletes	Cross-sectional	FFM, EEE, EI, EA, REE, bloods- TES, IGF-1, cortisol, ferritin, iron, TSH, T3, insulin Three factor eating questionnaire and short well-being questionnaire	EA 29.5 kcal.kg^{-1} FFM.day^{-1}, 66% below 30 kcal.kg^{-1} FFM.day^{-1} EA ↓ with ↑ EEE EI ↑ with ↑ cognitive restraint scores REE_{ratio} normal ↔ EA markers above/below 30 kcal.kg^{-1} FFM.day^{-1}	The threshold for EA in men may be lower than that identified for women.

Study	Population	Design	Markers	Results	Conclusions/Comments
Lane (Lane et al. 2021)	60 M recreationally trained	Observational cross-sectional	4-day food records, 7-day training logs. RMR, BMD, hormones, bone biomarkers.	Mean EA 28.7 kcal.kg^{-1} FFM.day^{-1} but all blood, bone markers and REE were normal. ↑ EA was significantly associated with ↓ total body BMD	Given RMR and EA findings were not aligned, the authors question the appropriateness of RMR as a proxy for EA. Male specific cut points for EA are needed.
Langan-Evans (Langan-Evans et al. 2021)	1 M combat sport athlete	Case study, 8 weeks	Body composition, EA, cardiac function, psychological state, endocrine markers, bone turnover, hydration, renal, lipids, liver and kidney function	EA ~ 20 kcal.kg^{-1} FFM.day^{-1} ↔ RED-S symptoms EA <10 kcal.kg^{-1} FFM.day^{-1} ↑ symptoms	A threshold for EA is required for male athletes.
Matt (Matt et al. 2021)	60 F 12 M adolescent cross-country runners	Cross-sectional	EI: Block FFQ EEE: mileage & Actiheart FFM: DXA or BIA LEA <30 kcal.kg^{-1} FFM.day^{-1}	M > EA than F (35.8 vs 29.6 kcal.kg^{-1} FFM.day^{-1}), fewer with LEA (30% M, 60% F)	Both M and F adolescent runners did not adequately fuel training.

Study	Population	Design	Markers	Results	Conclusions/Comments
Amorim (Amorim et al. 2021)	38 M, 63 F Dancers 47 M, 68 F Controls	Longitudinal, monitored over 3 years, measured annually	EI: 3-day food EEE: exercise records DXA: BMD & FFM LEA <30 kcal.kg^{-1} FFM.day^{-1}), IGF-1, BPAQ	Dancers - BMD ↓ all sites - IGF-1 ↔ - EA normal range but ↓ year on year (M & F) - ↓ fat and carbohydrate ↓ BMD with ↓ EA in F but not M	Raw BMD values were used, not z-scores. Whilst mean EA was within the normal range, the number of subjects falling outside of this range was not reported.
Moore (Moore et al. 2021)	14 M endurance trained athletes	Cross-sectional, assessed LEA v MAT components	LEA <30 kcal.kg^{-1} FFM.day^{-1} EI: 2x 7-day food record EE: exercise log, HR adjusted VO_2max ED: EDI-3 & EDI-3 SC DXA: BMD Blood: TES	Mean EA 27.6 kcal.kg^{-1} FFM.day^{-1} 35% ↑ risk for ED 64% LEA) EA ↔ high and low volume weeks TES, BMD were normal	Relatively high rates of LEA and risk of DE suggested but were present without other signs of MAT. EI was not adjusted for training load.
Moris (Moris et al. 2021)	44 M collegiate athletes, mixed sports	Cross-sectional, prevalence of MAT	EI: 3-day food records EEE: 7-day REE DXA: BMD & FFM Blood: T and F TES, SHBG, Insulin	15% LEA, 0% low BMD, 28% low TES, 80% low fTES, No sig correlations between EA, BMD, TES or fTES. Insulin negatively correlated with total and spine BMD.	EA assessment alone is insufficient to identify those with MAT. Low TES may not relate to BMD, insulin is worthy of further research attention.

Study	Population	Design	Markers	Results	Conclusions/Comments
Pritchett (Pritchett et al. 2021)	9 M 9 F para athletes, national level	Cross-sectional	LEA < 30 kcal.kg^{-1} FFM.day^{-1} LEAF-Q (F only) EDE-Q DXA: BMD, FFM Blood: TES, IGF-1, fT3	None LEA but high daily variation All M ↓ TES. BMD ↓ in several subjects but hard to interpret due to spinal cord injury. EDE-Q did not indicate risk in M	Qualitative and quantitative assessments did not align, the tools may need adjustment for the para population.
Stenqvist (Stenqvist et al. 2021)	44 M elite athletes	Cross sectional, descriptive	REE DXA: BMD & FFM Blood: TES, fT3, cortisol, lipids	Those with low RMR (RMR_{ratio} <0.9) had lower total TES. Leanness sport ↑rates of REDs surrogates	REDs surrogate markers 'clustered' in individuals, not always expressed in the same pattern. RMR was considered a useful proxy for EA assessment.
Tokuyama (Tokuyama et al. 2021)	19 M college rugby players	EA across a 2-week training camp	EI: photographic diary & weights, meals via cafeteria. EEE: METS for set training sessions	Negative EB, ↓ mass EA not calculated	The challenges of measured EA in a field setting were acknowledged.
Jesus (Jesus et al. 2021)	124 M 83 F cross country runners	Cross sectional- estimate prevalence of LEA using the LEAF-Q at a cross country running event.	Used LEAF-Q with modified scores (Using method by Slater) for M	LEA ↑ F v M (79.5% vs 54%).	The adjusted scoring and lack of male specific questions may contribute to the relatively lower prevalence in males, no proxy for male reproductive function. High prevalence of gastrointestinal symptoms (M and F) and menstrual dysfunction (F)

Study	Population	Design	Markers	Results	Conclusions/Comments
Intervention Studies					
Papageorgiou, (Papageorgiou et al. 2017)	11 M 11 W physically active	Randomised cross-over, counterbalanced design 2x 5-day trials of 1. LEA (15 kcal.kg^{-1} FFM.day^{-1}) 2. Control (kcal.kg^{-1} FFM.day^{-1}) LEA induced by a combination of diet and exercise (running).	Comparing BTM responses of M and F in response to LEA. PTH, IGF-1, fT3, insulin GLP-2, P1NP, β-CTX-I BTR (P1NP/β-CTX-I)	β-CTX-I ↑ and P1NP ↓ in women but not men in the LEA condition. Women also showed ↓ insulin and ↑ area under the curve for BTR. ↔ PTH, IGF-1, GLP-1 or fT3.	Bone turnover showed unfavourable changes in women but not men in response the LEA.
Koehler (Koehler et al. 2017)	6 M active	Repeated measures cross-over design with 4 interventions, 4 days each 1. LEA through diet 15 kcal.kg^{-1} FFM.day^{-1} 2. LEA through diet and exercise 15 kcal.kg^{-1} FFM.day^{-1} 3. Normal EA diet 40 kcal.kg^{-1} FFM.day^{-1} 4. Normal EA diet and exercise 40 kcal.kg^{-1} FFM.day^{-1}	• Weight and body composition (BIA) • Energy Availability assessment food records, exercise prescription • Total TES, fT3, insulin, IGF-1, Leptin, glycerol, BGL and FFA	LEA through diet restriction or a combination of diet and exercise ↔ TES, ghrelin, fT3, IGF-1, FFM ↓ weight, fat mass, leptin, insulin**	Reductions in leptin, insulin, weight and fat mass indicate LEA was achieved and was a significant stressor. The lack of change in IGF-1 and TES may indicate a higher threshold for disruption in men than in women. Limitations include method of assessment of body composition (BIA), use of a single fasting measure of hormone status rather than assessing pulsatility as in the female studies

Study	Population	Design	Markers	Results	Conclusions/Comments
Ishibashi (Ishibashi et al. 2020)	6 M distance runners	3 consecutive days of LEA (20 kcal.kg^{-1} FFM.day^{-1}) or neutral EA (45 kcal.kg^{-1} FFM.day^{-1})	Muscle glycogens, iron metabolism	LEA ↑ hepcidin and reduced muscle glycogen.	LEA may be a risk factor for iron deficiency in endurance athletes.
Stenqvist (Stenqvist et al. 2020)	22 M trained cyclists	4-week intensified endurance training, 3 high intensity interval sessions per week added to their normal training load	pre and post REE DXA Bloods Performance	- Performance ↑, peak power, VO_{2peak}, FTP - ↑ total TES, cortisol - ↓ fT3, RMR_{ratio} - ↔ TES:cortisol, cortisol:insulin	Short duration increases in training load may increase risk of RED-S in male athletes.
McKay (McKay et al. 2021)	28 M elite racewalkers	All subjects completed CON (high CHO & EA – 45 kcal.kg^{-1} FFM.day^{-1}) and either low carb high fat (LCHF) or LEA 15 kcal.kg^{-1} FFM.day^{-1}, 6 days per diet phase	IL-6, hepcidin, cortisol, glucose, WBC count	LCHF – ↑ IL-6 and hepcidin, WBC count and cortisol and lower BGL compared to baseline CON or LEA ↔ compared to baseline or between diets.	Short term restriction of CHO may have greater negative impacts on health than LEA.
Martin (Martin et al. 2021)	6 M recreationally trained	Repeated measures cross-over design, 2x 4 days LEA (15 kcal.kg^{-1} FFM.day^{-1}) with and without endurance exercise and 2x 4 control days with and without exercise.	EI, EEE, NEAT, mood state	LEA did not alter NEAT behaviour, but mood and perception of dietary restriction was influenced by whether the LEA was induced by diet + exercise or diet alone.	NEAT is varied and a large portion of the days TEE and it should be considered in EA calculations.

Study	Population	Design	Markers	Results	Conclusions/Comments
Murphy (Murphy et al. 2021)	7 M	Randomized, single blinded repeated measures crossover study. 3x 5-days 1. LEA (15 kcal.kg^{-1} FFM.day^{-1}), low protein (0.8g/kg) 2. LEA (15 kcal.kg^{-1} FFM.day^{-1}), high protein (1.7g/kg) 3. Control (kcal.kg^{-1} FFM.day^{-1}), protein (1.7g/kg) LEA induced by a combination of diet and exercise (cycling).	Height, weight, % body fat BIA, EEE & EI prescribed and formulated diet provided. Bone biomarkers- β-CTX-I, sclerositn, IGF-1, IGFBO-3, P1NP Leptin	Weight loss of ~2kg on both LEA diets. Leptin and P1NP ↓, and β-CTX-1 ↑ more in LEA than control. ↔IGF-1 A higher protein intake showed a trend to blunt rises in β-CTX-1	Negative changes to bone metabolism were induced by LEA and were not blunted by a high protein diet.

2.6 Tools for Identifying Low Energy Availability

Measurement of energy availability in field settings is challenging, ranging from the considerable time burden placed on the athlete and practitioner to the well-known issues in quantifying EI and EEE (Braakhuis et al. 2003, Westerterp 2009, Koehler et al. 2011). In addition, an assessment of EA at a given timepoint cannot identify whether it is of recent origin or reflects previous or chronic patterns. An athlete may have adequate EA in lower training blocks but may find it more difficult to meet their requirements during higher volume training (Burke et al. 2018b). Each sport has a unique culture and practices, and differences in issues underpinning the causation of LEA may alter its presentation or ease of diagnosis (Burke et al. 2018a). The development of tools to aid the identification of LEA would be highly valuable both for clinical care and facilitating research in this area (Mountjoy et al. 2018, Fredericson et al. 2021, Kuikman et al. 2021). This is especially the case for male athletes, who have been less well studied in terms of LEA. Tools include screening questionnaires and biomarkers that predate the development of serious impairments of health and performance and thus provide an opportunity for early intervention.

2.6.1 Screening Questionnaires for LEA

Although a variety of questionnaires have been used in research and clinical settings to identify signs and symptoms of LEA in male athletes (Sim et al. 2021), there has been little consistency in their approach. Furthermore, few of the questionnaires have been validated for the specific population with which they were used and indeed, the core construct (LEA) is in itself difficult to measure, providing challenge to the development of appropriate screening tools. A summary of these is presented in Table 4 and discussed in more detail below.

The Low Energy Availability in Female Athletes Questionnaire (LEAF-Q) was developed in a young female endurance population and is designed to identify those who may be at risk of LEA (Melin et al. 2014). It provides a screening tool that can be widely administered in large groups of athletes where a full assessment of energy availability is impractical. The prevalence of those identified at risk of LEA via this questionnaire is consistently high. Indeed, a recent study (Drew et al. 2017a) which screened female athletes during their preparation for the Rio Olympic Games reported that 53% achieved LEAF-Q scores indicative of a high risk of LEA. Importantly, this score was associated with an increased risk of illness. However, since the LEAF-Q was validated in a cohort of endurance athletes, adjustments may be needed for a more widespread application to diverse athlete populations. Rogers et al. found that the questionnaire could be used to “rule out” those at low risk of LEA but those scoring above the designated threshold would require further clinical assessment to identify LEA (Rogers et al. 2021b). A large-scale survey of 1,000 female athletes using a compilation of validated questionnaires found those with LEA (a surrogate of LEA determined by a flagged response

on one of three ED/DE questionnaires- BEDA-Q, ESP or a self-report of history) were more likely to be classified as having increased risk of menstrual dysfunction, poor bone health, metabolic issues, haematological detriments, psychological disorders and gastrointestinal dysfunction than those with adequate EA. Performance variables were also associated with LEA. Whilst both approaches have merit for female athletes, specific tools to identify LEA in male athletes have not been developed.

Some researchers have used questionnaires acting as proxies for LEA, including the exercise dependence scale (ExDS)(Hausenblas et al. 2002) and the Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn et al. 1994), and found associations with biomarkers of REDs in male endurance athletes (Torstveit et al. 2019). However, the EDE-Q is known to address eating and weight/shape control behaviours of females and may not fully address traits that more aligned with men (Mond et al. 2014). Others have repurposed the LEAF-Q or the Female Athlete Triad Cumulative Risk Assessment (FAT CRA) for male athletes, by removing the section on menstrual function and adjusting the scoring to account for a lower number of questions (Slater 2015) or by replacing the menstrual function questions with others addressing libido and morning erections (Kuikman et al. 2021). A large-scale study by Hackney et al, using a combination of validated questionnaires regarding physical characteristics, training and libido, demonstrated that higher training loads are predictive of lower libido. However, the focus of the questionnaire was the effect of training load on TES, and energy availability was not assessed in this study (Hackney et al. 2017). The Sport Specific Energy Availability Questionnaire and Interview (SEAQ-I) (Keay et al. 2018) is a questionnaire and clinical interview developed for male cyclists, but it relies on practitioner expertise for use and has only undergone content validity. Furthermore, it assumes LEA based on reported energy restriction and weight change. Poor validation processes also limit the REDs Specific Screening Tool (RST) (Foley Davelaar et al. 2020), since it was correlated against the pre-participation gynaecological examination (Parmigiano et al. 2014), another non-validated process which was developed for adolescent females without attention to sex differences in presentation of LEA symptoms. The Androgen Deficiency in Aging Males questionnaire (ADAM-Q) (Morley et al. 2000) has been used to identify male athletes with changes to their reproductive function in association with their training (Logue et al. 2021) but, as with female athletes, reproductive dysfunction may have causes outside of LEA which are not addressed. The Dance Specific Energy Availability Questionnaire (DEAQ) (Keay et al. 2020) utilises questions from previously validated questionnaires including LEAF-Q and ADAM-Q (Morley et al. 2000) as well as questions used in REDS-CAT (Mountjoy et al. 2015) and SEAQ-I (Keay et al. 2018). However, these have not been validated to identify LEA in male athletic populations either separately or in the current format. Other researchers (Kraus et al. 2019) have used a modified version of the FAT CRA tool which removed the questions relating to female reproductive function to successfully assess risk of bone stress injury.

Table 4: Questionnaires relevant to the development of a LEA screening tool for male athletes

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
LEA/FAT/MAT/RED-S						
De Souza (De Souza et al. 2007)	Determine the association between drive for thinness (DT) and LEA in active women	43 F active 9 F control	REE fT3, ghrelin, leptin, insulin EDI- DT EI EEE	High DT had low REE, fT3, higher ghrelin than normal DT	DT correctly discriminated individuals with signs of chronic LEA.	DT may be a useful tool to identify LEA in females.
Mencias (Mencias et al. 2012)	Summary of FAT questions included in PPE screening in US relative to the recommendations of the FAT coalition.	347 NCAA Div I universities	Evaluations of screening practices and preparticipation surveys to identify the FAT	100% of universities required a PPE for new athletes, but 32% for returning athletes. 9% included 9 of 12 recommended FAT questions and 44% had less than 4 of 12.	Screening by NCAA universities for FAT is inadequate in many cases.	Questionnaires need to short and easy to administer to be taken up by large scale organisations that may benefit from using them.
Melin (Melin et al. 2014)	Validation of a questionnaire to identify risk of LEA and FAT conditions in female endurance athletes (LEAF-Q)	84 F endurance athletes	REE, EA, EEE, EI, ED, DXA FFM and BMD, EDI-3, EDE-16 validity assessed by testing self-report again measured data for variables- EA, menstrual function and bone health	78% sensitivity and 90% specificity to correctly classify EA, bone health or reproductive function.	LEAF-Q can be used as a screening tool for LEA as a complement for DE screening.	Validated in an endurance population.

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
DeSouza (De Souza et al. 2014)	Questionnaire developed as part of the return to play model for the Female Athlete Triad – Cumulative Risk Assessment	-	LEA with or without DE/ED BMI Menarche Menstrual status BMD Bone Stress injury	Risk stratification using risk factures identified in the literature as have formed the basis for the Female Athlete Triad.	Guidance as to suitability to return to sport. Not validated.	Using a cumulative risk allows risk to be considered across three key areas.
Mountjoy (Mountjoy et al. 2014, Mountjoy et al. 2015)	A clinical tool designed to stratify risk of REDs – REDS CAT	Not validated	Red, amber, green categorisation across several REDS categories to guide assessment and suitability for return to play.	Provides a framework to clinicians to assess risk.	At present it is a clinician's tool which allows assessment of both males and females but not a validated screening tool for self-report.	Risk stratification.
Slater (Slater 2015)	Estimate prevalence of those at risk of LEA in recreational NZ athletes using a combination of Eating Disorder Inventory – 3 (EDI-3) and LEAF-Q	61 M 109 F Recreational athletes	Questionnaire responses, BMI	33.5% of participants were classified as at risk of LEA with more females (44.9%) than males (13.1%)	Whilst LEA prevalence was assessed in males no substitution was made for reproductive function and the markers for males and females with LEA may not be the same.	A marker of disruption to male reproductive function needs to be included in the questionnaire.

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Barrack (Barrack et al. 2017)	Survey to identify predict low bone mass in male adolescent athletes	51 M adolescent runners 18 controls	Sport participation, nutrition, stress fracture history, DXA BMD, body composition.	Runners had lower body weight, BMI, % expected weight, spine BMD z score. Predictive factors included low weight, high mileage, low calcium intake.	Risk factors were cumulative when predicting low BMD. Those with 3-4 risk factors has an 80% chance of low BMD.	Supports a scoring system based on multiple areas
Keay (Keay et al. 2018)	Evaluate a sport specific EA questionnaire combined with clinical interview for assessing risk of REDs (SEAQ-I)	50 M road cyclists	SEAQ-I responses to allocated as chronic LEA, Acute LEA, or adequate EA. DXA BMD and FFM Endocrine markers- TES, T3, Vit D, calcium, alkaline phosphatase Content validity assessed	SEAQ-I identified 28% as having LEA Subclinical low status for Vit D, TES, fT3 Low lumbar spine BMD 44% and most associated with SEAQ-I responses.	The authors found the questionnaire to be effective at identifying LEA in cyclists and to have an association with bone, endocrine and performance consequences of REDs	EA was not measured and the questionnaire has not been validated to identify LEA. Performance aspect unclear. Categorisation Clinical skills are of value in LEA assessment

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Ackerman, 2019 (Ackerman et al. 2019)	Online questionnaire to identify LEA and REDs outcomes LEA: assessed via BEDA-Q, ESP, self-report of ED or DE history	1,000 F athletes	1. Menstrual function 2. Bone health- bone injury or z<-1 3. Metabolic- RMR 4. Haematological- anaemia 5. Endocrine function 6. Growth & development 7. Psychological 8. Cardiovascular 9. Gastrointestinal 10. Performance	LEA ↑ menstrual dysfunction, endocrine metabolic or haematological abnormalities, psychological disorders, cardiovascular and gastrointestinal symptoms ↓ bone health & performance ↔ between groups for growth and development or immunological function, injury risk.	LEA is associated with most of the health and performance consequences outlined in the REDs model.	Injury and illness may not be predictive in female athletes.
Kraus 2019 (Kraus et al. 2019)	To determine whether the FAT CRA tool could predict bone stress injury (BSI) in male distance runners	156 M	LEA/DE (based on PPE questions) BMI BMD BSI Baseline and change over time	27% sustained BSI over ~ 2 year follow up. CRA score was associated with a 37% ↑ risk of BSI. Prior BSI was predictive, but no other single factor was. LEA and DE/ED were uncommon at baseline.	Combining risk factors was most strongly predictive of BSI.	EA was poorly defined in this assessment. CRA responses with menstrual function section removed.

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Torstveit (Torstveit et al. 2019)	Exercise dependence scale (EXDS) and EDE-Q as potential proxies for RED-S	53 M endurance athletes	ExDS, EDE-Q DXA body composition REE EI, EEE Blood: hormones and glucose	↑ EXDS ↑ negative EB EXDS correlated with EDE-Q global score, restrained eating, weight concern. Cortisol correlated with EXDS total score, lack of control and tolerance subscales. EXDS total score and subscales for withdrawal and tolerance were negatively correlated with fasting BGL Intention effect was negatively correlated with TCR and positively with cortisol:insulin	ExDS and EDE-Q scores in M associated with biomarkers of RED-S. Exercise dependence with or without ED might contribute to RED-S	Cortisol, insulin and TES are biomarkers of interest. Used alongside identification of LEA these may be helpful.
Keay (Keay et al. 2020)	Investigate correlates of LEA in male and female dancers	225 F 22 M dancers	Dance specific Energy Availability Questionnaire (DEAQ) formed from LEAF-Q, SEAQ-I, REDS CAT and ADAMS-Q	DEAQ used a derived scoring system to identify markers of LEA in dancers – 57% in F, 29% in M, but awareness of REDs was low (29%).	Further validation of DEAQ and education is required in this population.	M sample small, M specific question limited. Observational questionnaire, content validity only.

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Foley Davelaar (Foley Davelaar et al. 2020)	Determine concurrent validity of REDs specific screening tool (RST) and the validated Pre-participation gynaecological examination (PPGE)	39 F	PPGE Eating disorder screen	The questionnaire was considered valid given it was correlated with PPGE ($r=0.697$, $P<0.001$). The PPGE was not itself validated and the RST uses questions from it as well as correlating responses to it. A questionnaire is proposed for males however no investigation was undertaken in male populations.	No conclusions can be drawn from this.	The validation process undertaken was not adequate.
Rogers 2021 (Rogers et al. 2021b)	Exploring the ability of the LEAF-Q to detect conditions relating to LEA in a mixed cohort.	75 F	LEAF-Q score, RMR, SCOFF, DXA body comp and BMD Blood metabolic and reproductive hormones.	55% scored above 8 on the LEAF-Q. Injury and menstrual function scores identified low BMD and menstrual dysfunction. The gastrointestinal score did not identify markers of LEA.	The LEAF-Q can be used to rule out those at risk of LEA but not rule in.	Low specificity meant that Low specificity meant that it could not rule in subjects with LEA, only rule out.

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Kuikman 2021 (Kuikman et al. 2021)	Examining the relationship between exercise dependence (ExD), disordered eating (DE) and LEA	257 M 642 F athletes	Categorised by risk of ExD, DE, LEA or controls. LEA assessed by LEAF-Q, males were asked about reproductive dysfunction in lieu of menstruation.	Male athletes with disordered eating, were more likely to report suppression of morning erections (OR = 3.4; $p < 0.0001$), ↑ gas and bloating (OR = 4.0–5.2; $p < 0.002$), previous bone stress fracture (OR = 2.4; $p = 0.01$) and ≥22 missed training days due to overload injuries (OR = 5.7; $p = 0.02$).	Exercise dependence ↑ risk of LEA when it co-occurred with disordered eating. M and F athletes show similarities in expression of LEA, M specific assessment tools are required. Recreational athletes were more at risk than international level athletes.	Questions regarding morning erections, gastrointestinal function, bone stress and injury may be important in M athletes with LEA.
Luszczki, 2021 (Luszczki et al. 2021)	Use of LEAF-Q and associated measure in an adolescent female football setting	34 F adolescent football players	LEAF-Q score DXA BMD & FFM Weight, height, BMI REE, EI	2/3 of participants were classified as at risk for FAT by the LEAF-Q scores. EI was lower in those at risk of LEA but no other measured area.	LEAF-Q scores were high but not correlated with FAT. EA or DE were not measured.	It is important to use a tool validated for the population.
Goldstein, 2021 (Goldstein et al. 2021)	Determine the relationship of the ePPE with the FAT CRA	239 F	Athletes categorised as low, moderate or high risk by FAT CRA. Logistic regression was used to explore association with ePPE responses.	ePPE questions were not associated with FAT CRA risk stratification.	ePPE was not sensitive enough to detect triad risk	Issue using self-report ED questions as a proxy for triad risk and the need for sport specific questions.

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Eating Disorders/Disordered Eating/Exercise Dependence						
Black (Black et al. 2003)	Development of a physiologic screening test (PST) for eating disorders/disordered eating in athletic females	148 F collegiate athletes	EDE 12.0D for diagnosis EDI-2, Bulimia Test-Revised and the proposed PST were evaluated. Target criteria sensitivity > 80%, specificity >75%	PST performed better than the existing questionnaires relative to the gold standard interview.	A physiologic screening test may reduce response bias and it may be more easily included in preparticipation examinations	Method of questionnaire evaluation against clinical assessment, sensitivity and specificity cut points identified.
Hildebrandt (Hildebrandt et al. 2010)	Development of a male specific body checking questionnaire (MBCQ) 1. Development of questionnaire 2. Confirmatory factor analysis 3. Test-retest reliability	Study 1: 196 M 146 F Study 2: 549 M Study 3: 27M	Body checking questionnaire EDE-Q EDI- perfectionism scale Muscle Dysmorphic Inventory	The questionnaire showed the need for gender specific questions in relation to the valued physique attributes	Further research needed to include male specific forms of body checking and assess the relationship between self-report and behaviour for body checking.	The validation process is similar to that employed with LEAM-Q despite different topics.
Schaefer (Schaefer et al. 2018)	Validation of EDE-Q for the detection of eating disorders in male populations	205 M ED 205 M students	Questionnaire scores plus clinical diagnosis	Sensitivity and specificity of 0.77	Preliminary support for EDE-Q among males but further evaluation suggested to ask more male specific questions.	The statistical approach to derive cut points, sensitivity and specificity is similar to LEAM-Q

Study	Purpose/Design	Population	Markers	Results	Conclusions	Points to consider for LEAM-Q
Other						
Hackney (Hackney et al. 2017)	Assess aspects of endurance training and sexual libido in health males using an online Q	1077 M	Training volume, intensity, libido. Questionnaire derived from International Physical Activity Questionnaire, Baecke Questionnaire, ADAM-Q, Sexual Desire Inventory 2 and AMS	Libido showed an inverse relationship with training duration/intensity	Men engaged in a high volume or intensity of exercise over years are more likely to show a lower libido.	Training characteristics and libido might be important factors to consider for LEA in men.
Morley (Morley et al. 2000)	Validation of a Questionnaire to screen for androgen deficiency associated with aging (ADAM-Q).	316 M 40-62 y	TES and Free TES, LH Yes/no responses to (ADAM-Q)	25% had low free TES. The ADAM-Q had 88% sensitivity, 60% specificity	The questionnaire performed well at identifying the symptom complex associated with low TES is men > 40y	Require assessment for use young M and athletes.
Logue (Logue et al. 2021)	Online questionnaire to determine prevalence of impaired reproductive function in male athletes (EHMC) using a modified version of the ADAM-Q	589 M active	Reproductive function Injury Illness Diet habits	Risk of EHMC was identified in 23.3% of the sample. Associated with ↑ rate of injury and time away from training and competition. ↔ dietary habits, elite status	More research in males is required, evidence of DE/ED and health consequences of REDs.	A lower sex drive was associated with injury risk and may indicate LEA.

2.6.2 Resting Metabolic Rate

Resting metabolic rate (RMR) is the rate at which energy is used at rest to run vital body functions such as breathing, thermoregulation and reproductive function and is , expressed as kcal or kJ per minute (Haugen et al. 2007). Resting energy expenditure (REE) is RMR expressed over a 24-hour period. The measurement of RMR might be undertaken to detect adaptive thermogenesis, reflecting a reduction of REE in response to energy restriction to defend the body's energy stores (Grande et al. 1958). Early examples include the Minnesota Starvation Experiment, in which decline and recovery of metabolic rate with caloric restriction and subsequent refeeding was demonstrated (Grande et al. 1958). This protocol was revisited, with 32 men prescribed overfeeding, underfeeding and refeeding sequentially, alongside close monitoring of body composition and RMR. REE decreased by 266 kcal/day, attributed to loss of lean tissue and adaptive thermogenesis with reduced heart rate, insulin secretion and body fluid balance (Muller et al. 2015). Other populations with reduced energy intake, such as patients with anorexia nervosa have shown depressed RMR when compared with those who had recovered from their illness and to healthy controls (Platte et al. 1994). Further, women with higher scores for restrained eating or drive for thinness have lower RMR than those with lower scores (Platte et al. 1996, De Souza et al. 2007). Lowered RMR has been suggested as a clinical marker of LEA in female athletes (Kaufman et al. 2002, Melin et al. 2015).

Female lightweight rowers who dieted (McCargar et al. 1993), dancers with menstrual irregularity (Myburgh et al. 1999) and female endurance athletes with LEA (Melin et al. 2015) or menstrual irregularity (Myerson et al. 1991, Melin et al. 2015) have been shown to have lower RMR than their normal counterparts. Male athletes have less frequently been investigated but similar findings have been identified. Low REE in male athletes has been reported in association with inadequate EI (Thompson et al. 1993), LEA (Lee et al. 2020, Taguchi et al. 2020) and during intensified training periods where EI was not increased accordingly (Woods et al. 2017, Stenqvist et al. 2020). In contrast, competitive recreationally trained male endurance athletes assessed for EA and associated biomarkers had normal RMR, despite a group mean EA indicating LEA (28.7 ± 13.4 $kcal.kg^{-1}$ $FFM.day^{-1}$) (Lane et al. 2021). Given there were also no other perturbations of biomarkers including thyroid hormones, TES and BMD, it seems possible that the participants were not in a state of LEA and may support the argument that male athletes are more resilient to LEA and require a sex specific threshold (Koehler et al. 2016).

The use of RMR as an indicator for LEA in athletes presents methodological challenges in both the measurement and interpretation of data. The standardisation of training and diet in the pre-test period is important in separating acute effects from chronic adaptation (Sjodin et al. 1996, Compher et al. 2006). However, this may not be practical to implement in the real-life training programs of high-performance athletes. The Interpretation of measurements, expressed per kg of fat free mass,

is also difficult since it assumes the body composition characteristics of sedentary population. Meanwhile the physique of athletes may range from extremes in both the total amount of FFM and its breakdown into organs with relatively high metabolic rates and muscle mass with lower metabolic rates (Muller et al. 2002, LaForgia et al. 2004). Therefore, assumptions regarding the mean metabolic characteristics of FFM may be invalid in such special populations.

In the currently available research, methods to assess RMR have included statistically adjusting REE for body mass or FFM to compare participant groups in research trials (Myerson et al. 1991), expressing REE relative to FFM (Kaufman et al. 2002, De Souza et al. 2008) and using a ratio of measured to predicted RMR (De Souza et al. 2007). RMR prediction equations vary in the accuracy with which they predict RMR for athletes which is logical given the variety of populations they have been developed for. Thompson et al (Thompson et al. 1996) found only the Cunningham equation (Cunningham 1980) was acceptable as an estimate of measured RMR in a mixed athlete cohort. An RMR_{ratio} of <0.9 has been proposed and widely adopted as a point at which RMR is supressed and represents insufficient EI (De Souza et al. 2007) however the cut point has not been tested in men and may vary with the prediction equation used (Fredericson et al. 2021, Sterringer et al. 2022).

Other factors relating to the timing of EI relative to exercise rather than total EI may affect RMR. In one study, male endurance athletes with a supressed RMR did not show differences in EA or 24-hour energy balance but had larger discrepancies in the timing of energy intake over the day in relation to exercise periods, leading to larger single hour deficits in EB than those with normal RMR (Torstveit et al. 2018).

2.6.3 Blood Biomarkers of LEA in Male Athletes

Several blood parameters are altered in LEA (reviewed in (Elliott-Sale et al. 2018, Dipla et al. 2021). Key parameters and how these differ in males than females are outlined below.

Thyroid Hormones

Triiodothyronine (T3) is a hormone produced by the hypothalamus that is important for growth, reproduction, and metabolism (Elliott-Sale et al. 2018). There are consistent observations that it is reduced in women with functional hypothalamic amenorrhoea (Loucks et al. 1992) and anorexia nervosa (Warren 2011) and in controlled experiments inducing LEA (Loucks et al. 1994). Meanwhile in males, low T3 has been seen in anorexia nervosa (Skolnick et al. 2016), in military settings involving energy restriction combined with high levels of physical activity and other physical stress (Opstad et al. 1984, Friedl et al. 2000) and in distance athletes with higher RED-S CAT scores (Heikura et al. 2018a).

Testosterone

TES is an androgenic, anabolic hormone, produced in the testes in response to luteinizing hormone as part of the hypothalamic pituitary testicular axis (Vingren et al. 2010, Alves et al. 2020) (Figure 4). TES is strongly bound to sex hormone binding globulin and although the measurement of free or unbound TES is potentially important when understanding changes to serum levels in athletes, this remains a cause for debate (Keevil et al. 2019).

There are consistent reports of reduced TES concentration in endurance trained male athletes (Hackney et al. 1988, 1990, Wheeler et al. 1991, Hackney et al. 1998, Alves et al. 2020), a condition described as EHMC (Hackney et al. 2005). Eating disorders (Sabel et al. 2014, Silla et al. 2021), military studies of energy restriction (Friedl et al. 2000), fasting (Cameron et al. 1991) and low energy availability (Heikura et al. 2018a, Nattiv et al. 2021) have been associated with low TES in men as described in more detail in 2.5.1 Energy Availability in Male Athletes.

Cortisol

Cortisol is secreted by the adrenal cortex under the control of the hypothalamic pituitary axis in response to prolonged exercise, stress or energy restriction acting as a catabolic hormone (reviewed in (Elliott-Sale et al. 2018, Alves et al. 2020)). Increased cortisol concentrations have been seen in protein energy malnutrition (Smith et al. 1975), in female athletes with amenorrhoea (Ding et al. 1988), men with anorexia nervosa (Skolnick et al. 2016) and military studies including energy restriction, high levels of physical activity and other stressors (Friedl et al. 2000). Scenarios of increased cortisol response in male athletes include judokas undergoing acute weight loss (Degoutte et al. 2006, Abedelmalek et al. 2015), athletes with greater time in negative energy balance (Torstveit et al. 2018) and intensified training periods where energy intake was not increased to match the training load (Stenqvist et al. 2020). Conversely, however, no differences were seen between endurance runners and controls (Hackney et al. 1988) or those with EHMC or without (Hooper et al. 2017). Although cortisol appears to be a potentially useful biomarker of LEA, the separate effects of training and stress, and the divergence between acute and chronic responses (Viru et al. 2004) mean that further research is needed to understand its utility.

Testosterone:Cortisol Ratio

The free testosterone:cortisol ratio (fTCR) was originally described by Aldercreutz et al, as a marker of training stress and catabolism. It was noted that short term, intense physical activity leads to increased cortisol and decreased total TES, with a counterbalanced increase in the percentage of free TES. Following prolonged exhaustive exercise, both free and total TES are reduced. The syndrome of overstraining, now described as overreaching, was first identified on the basis of a 30% decrease in fTCR (Adlercreutz et al. 1986). Since then, the concept has been used to monitor training fatigue in sports such as soccer (Banfi et al. 2006, Hammami et al. 2017), endurance running (Luccia et al. 2018)

and rowing (Vervoorn et al. 1992, Jurimae et al. 2001, Ramson et al. 2009). Variations in the use of free and total TES, the unit of measurement reported, whether saliva or serum samples were collected, and other aspects of methodology make it difficult to gauge the utility TCR as a biomarker difficult. There is also controversy as to whether a reduced ratio is indicative of maladaptation or a normal and required response to training (Viru et al. 2004).

In addition to providing a metric of training stress monitoring, TCR has been reported in the nutrition literature as a marker of a catabolic state. Shared pathways for both symptoms and causation have been noted for the overtraining spectrum and LEA (Stellingwerff et al. 2021) and TCR may be a helpful LEA biomarker. However, Lane et al noted that fTCR decreased by 43% on a low carbohydrate diet compared with 3% on an adequate carbohydrate diet in response to a training stimulus (Lane et al. 2010). TCR was reported in athletes assessed for exercise dependence but was not different between those who scored higher or lower on the EXDS (Torstveit et al. 2019). In cyclists who completed a 4-week intensified training programme which induced a reduction in REE, no change in either fTCR or tTCR was identified (Stenqvist et al. 2020).

Insulin

Insulin regulates the storage of energy and is reduced in LEA to allow more substrate availability. Insulin also has a role in GnRH signalling and has been associated with LH activity (Elliott-Sale et al. 2018). Reduced insulin levels have been associated in men following fasting (Chan et al. 2003), energy restriction (Grande et al. 1958, Friedl et al. 2000, Maestu et al. 2010, Muller et al. 2015) and with induced LEA (Koehler et al. 2016). In the latter study, perturbations of insulin and leptin were the only indicators of a the LEA response.

Cortisol:Insulin Ratio

Loucks (Loucks 2013) proposed the use of the cortisol:insulin ratio as a marker of accelerated proteolysis, based on findings of an earlier study (Loucks et al. 2003b) in which there was a stepwise increase with graded decreases in EA. This is further supported by an observational study (Laughlin et al. 1996) showing higher a ratio in amenorrhoeic athletes compared to eumenorrheic athletes and sedentary controls. The observation that glucose infusion during exercise blunts and increase in cortisol:insulin suggests that it reflects fuel status during exercise than exercise per se (MacLaren et al. 1999). The cortisol:insulin ratio is relatively untested in the LEA literature, with only one recent study of EXDS examining its utility as a marker. Here the researchers reported no differences in the cortisol:insulin ratio between those with and without EXDS (Torstveit et al. 2019).

Insulin like growth factor-1 (IGF-1)

IGF-1, considered essential for the normal growth of bone as well as maintenance of bone mass in adulthood, is associated with fracture risk in older people (reviewed in (Vandenput et al. 2012)). It

has been correlated with energy deficits in some but not all studies. For example, there was a decrease in IGF-1 when women were exposed to 5 d of LEA in a laboratory situation (Loucks et al. 1998b, Loucks et al. 2003b) however this was not seen in a free-living scenario of LEA exposure in male athletes (Koehler et al. 2016). In protocols involving more severe energy restriction combined with other stressors in military settings (Friedl et al. 2000, Nindl et al. 2007) IGF-1 declined. Male athletes in sport settings such as cycling (Geesmann et al. 2017) and body building (Maestu et al. 2010) also showed a decline in IGF-1 response to insufficient energy intake.

Lipids

Extensive weight loss and anorexia nervosa have been associated with changes in blood lipids, namely an increase in cholesterol, both LDL and HDL ((Ende 1960), reviewed (Stone 1994)). This may be secondary to changes in either thyroid hormones, IGF-1 or a combination of both (Prewitt et al. 1992, Bogner et al. 1993).

Young healthy males undertaking an 8-week US Army Ranger course underwent 4 cycles of restricted energy and showed an ~140% increase in total cholesterol from both HDL and LDL fractions (Friedl et al. 2000). Marniemi et al imposed a severe energy restriction combined with walking of 344km over 7 days in both men and women and found a 30-40% reduction in total cholesterol, but a tendency for HDL cholesterol to increase. Obese females on very low-calorie diets showed an initial fall in cholesterol (month 1-2 of weight loss) followed by an increase that persisted until weight loss was ceased. The increase was attributed to mobilisation of adipose cholesterol stores (Phinney et al. 1991). Higher total cholesterol has also been seen in female endurance athletes with LEA compared to those with normal EA (Melin et al. 2015). Such metrics have been less frequently measured in males, but similar differences have identified (Langan-Evans et al. 2021, Stenqvist et al. 2021).

Blood Glucose

Fasting blood glucose levels (BGL) are lower in females with LEA. Early EA studies showed that participants with exposure to induced LEA of 13 Cal/kg LBM had lower BGL overnight, during waking and in response to feeding (Loucks et al. 1998b) compared to those who consumed adequate energy. This outcome was further replicated in an EA dose-response study (10, 20, 30 or 45 kcal.kg LBM^{-1}.day^{-1}), with findings of a generally more pronounced effect on blood glucose with greater degree of LEA (Loucks et al. 2003b). Similarly female endurance athletes with a LEAF-Q score of eight or more (indicating a high risk of LEA) had lower fasting BGL than those with lower scores (Melin et al. 2014).

In terms of studies of male athletes, fasting BGL was decreased when participants were exposed to LEA of 15 kcal.kg^{-1} FFM.day^{-1} FFM (Koehler et al. 2016). Meanwhile, male endurance runners who completed 3 days of endurance training under either LEA (19 kcal.kg^{-1} FFM.day^{-1}) or adequate EA (53 kcal.kg^{-1} FFM.day^{-1}) in a randomised crossover design were found to have reduced muscle glycogen in

the LEA arm, but although BGL decreased from day 1 to 4, overall differences in BGL between LEA and adequate EA were not significant (Kojima et al. 2020).

2.6.4 Blood pressure

Within the clinical literature, hypotension has been associated with anorexia nervosa in females (Katzman 2005) and males (Sabel et al. 2014, Skolnick et al. 2016) and those with severe energy restriction (Muller et al. 2015). Low systolic BP has been identified in exercising women with long term hypoestrogenism compared with normally menstruating exercising and sedentary females (O'Donnell et al. 2007), in dancers (Staal et al. 2018) and endurance athletes with LEA (Melin et al. 2015). Low RMR has also been associated with low systolic blood pressure (Sriram et al. 2014). Whilst low BP has only rarely been assessed in male athletic populations with LEA (Staal et al. 2018), there is a good basis to suggest it would be a worthwhile biomarker.

2.7 Exercise, Calcium and Bone Remodelling

Despite the generally positive effect of sport on BMD, athletes still experience sub-optimal bone health which may manifest in more immediate consequences for injury risk and/or longer-term issues of osteoporosis after retirement (Kohrt et al. 2004). Bone stress injuries can significantly affect an athlete's ability to train consistently and perform at their best. As such, service teams in elite sport frequently target strategies for prevention or harm minimisation in relation to sub-optimal bone health.

Observations of reductions in BMD over the course of a season or career, such as those reported in female (Sherk et al. 2014) and male cyclists (Barry et al. 2011) as well as in basketball players (Klesges et al. 1996), have prompted further investigation into causation. Whilst LEA is often an important contributor, low BMD in athlete groups has been seen in the absence of any other markers of this issue (Stenqvist et al. 2021). Early research postulated that dermal calcium losses associated with sweating may be a trigger for bone loss in athletes. Although adequate daily calcium intake is generally recognised as a key factor for bone health (2.4.2 Calcium, Vitamin D and Vitamin K), the timing of calcium intake, particularly prior to exercise, may be just as important.

Exercise and Calcium- Field Studies

Klesges et al undertook the first investigation of the effect of pre-exercise calcium intake on bone health. Bone mineral content (BMC) was measured in 11 male college basketball team over two seasons, the first without and the second with calcium supplementation. Dermal calcium losses, which averaged 422 mg per training session, were suggested to be a cause of ongoing bone loss and stress fracture risk. In the second season, supplementation was graded to match individual dermal

calcium losses (between 600-1800 mg/d) and provided as a combination of a supplement (consumed freely over the day) and fortified drink (consumed at training sessions and prior to games). While, a 6.1% loss of BMC was recorded over the first season, a 2% recovery was observed over the season involving calcium supplementation. (Klesges et al. 1996). Whilst improvements to BMC were seen in this study, it is not possible to separate the impact of providing additional calcium per se from its specific timing of intake prior to exercise.

Testing the hypothesis of the contribution of dermal calcium loss to bone loss, 42 male firefighters were monitored periodically over 4 months of training for sweat calcium losses, dietary calcium intake and measures of BMC. BTM (OC, PC1P and CTX) were measured before and after the training block. Although calcium intake was below recommendations in approximately half the subjects, bone measures remained stable except for increases in hip BMD and BMC and whole-body BMC. In spite of large interindividual variations in BTM, there was an overall increase in PC1P, stable concentrations of OC, and a decrease in CTX, suggesting a pattern towards bone formation. Sweat calcium concentration was not related to any other variable. The authors concluded that physical activity with a high sweat rate did not impair the bone health of participants (O'Toole et al. 2000).

Meanwhile, Barry et al investigated 20 male competitive cyclists, randomised to receive 1500mg or 250mg calcium daily over the course of a training year. Dermal calcium losses were measured at baseline and the study mid-point, while BMD was assessed at four points during the season. There was a 1.5% reduction in total hip BMD over this time, with spine BMD showing a non-significant trend to a loss of a similar magnitude. There were no differences in BMD change across any sites between the high or low calcium group. Higher dermal losses were associated with lower starting hip BMD but not with supplementation group or change in BMD (Barry et al. 2008).

Together, whilst the challenges of field studies are recognised, the current literature does not provide clear support that dermal calcium losses contribute to bone loss in athletes or that calcium supplementation is beneficial.

Acute Calcium Intake- Laboratory Studies

A series of studies have investigated changes in bone turnover in response to exercise and the possible impact of calcium on the response (Table 5). Barry et al (2007) observed an exercise-associated perturbation of bone remodelling in male cyclists (n=20) who completed two hours of moderate intensity cycling. PTH and iCa increased in response to exercise but neither dermal calcium loss nor change in iCa were related to PTH levels (Barry et al. 2007). Acute calcium supplementation, intended to counter dermal calcium losses during exercise, was investigated in separate studies involving elite male triathletes (Guillemant et al. 2004) and cyclists (Barry et al. 2011). A cycle ergometer exercise protocol of ~1 hour was undertaken, with calcium-rich mineral water being

consumed prior to exercise to provide a high or low calcium condition. In the triathletes, the calcium condition partially suppressed the rise in PTH and completely suppressed the rise in β-CTX-I seen with the control trial (Guillemant et al. 2004). The cyclists completed a third trial in which the calcium supplementation was provided during rather than before the exercise session (Barry et al. 2011). Although PTH was reduced by calcium supplementation prior to exercise, there was no effect on iCa or β-CTX-I. Taken together this suggests that pre-exercise oral calcium supplementation maintains iCa and attenuate rises in PTH. The contrast in β-CTX-I responses between the two studies may be a result in differences in timing of consumption of calcium (60 mins vs 20 mins prior). This theory is supported by work from Sherk et al, where calcium supplementation 30 minutes prior to a 35km cycling time trial attenuated the decline in iCa, a trend to reducing the rise in PTH but no change in β-CTX-I (Sherk et al. 2017).

Studies of exercise-related changes to calcium homeostasis are not limited to male athletes or to supplemental forms of calcium. Female cyclists (n=32) undertook an investigation of the effect of pre-exercise calcium from food sources (dairy), by consuming a meal with either high (1,200mg+) or low (<50mg) calcium content, 2 hours prior to completing a 90 min cycle session. PTH and β-CTX-I were increased by exercise in both trials but attenuated by the calcium-rich meal. Dermal calcium losses were also measured but were not correlated to bone turnover markers or BMD measures. This study demonstrated that dietary calcium can be used in place of supplemental calcium with similar effect, when considerations around the timing of ingestion to allow gut release of calcium are undertaken (Haakonssen et al. 2015). Studies in older individuals with lower levels of fitness also show similar responses to both calcium and exercise (Shea et al. 2014, Wherry, 2019 #8667, Wherry, 2021 #8825).

A protocol involving an intravenous infusion of calcium or saline was developed to focus on the drop in serum iCa as the trigger for changes in bone turnover, and the time course of its occurrence during exercise. Cycling sessions lasting 60 mins at 80% HR_{max} were monitored, with increases in PTH and β-CTX-I being observed in both the calcium and saline trials, but with an attenuation of increase in the calcium-infused cohort. An increased frequency of blood sampling during the exercise protocol demonstrated that the drop in iCa occurred as early as 15 minutes into the exercise bout (Kohrt et al. 2018), making the hypothesis of accumulating dermal sweat losses over the session an unlikely cause. A separate study was undertaken to compare bone turnover markers in cool and warm conditions leading to different sweat rates. This protocol found that although sweat losses were 50% higher in the warm conditions, iCa, PTH and CTX were not different. In both trials iCa dropped early in exercise, ahead of significant dermal losses. The authors concluded that dermal losses are not the primary trigger for changes to bone turnover during exercise (Kohrt et al. 2019).

In summary, exercise has been shown to disrupt calcium homeostasis, likely triggered by a decline in iCa which results in an increase in PTH. The reason for the drop in iCa is still to be determined but give the early onset and lack of relationship to the magnitude of sweat calcium losses, other causes need to be identified. Over time this exercise-associated change in bone turnover might be a contributor to the bone loss seen in sport.

Multiple Exercise Sessions

Whilst pre-exercise calcium attenuates markers of bone breakdown in athletes following a single exercise bout, it is noted that many athletes undertake several training sessions each day. Relatively few studies have focused on the effect of multiple exercise bouts on bone turnover markers. The effect of recovery duration between two bouts of running was investigated using either a short (3 hour) or long (23 hour) recovery period. Patterns of bone turnover markers were consistent between bout one and two and were not influenced by recovery duration (Scott et al. 2013). In contrast, a very short recovery window (40 mins) blunted the PTH response to a second exercise bout in comparison to completing the session as one continuous block (Bouassida et al. 2003).

A simulated four-day cycling race, consisting of three hours of riding per day, reported an elevation of post-exercise PTH on days one, two and four and β-CTX-I increases post exercise on days one and two but decreases on the final two days. Pre-exercise β-CTX-I and BAP were elevated relative to day 1 on all other days suggesting increased bone remodelling in response to repeated heavy training loads (Oosthuyse et al. 2014). In contrast, a field study monitoring pro-cyclists competing the *Giro D'Italia* showed PTH levels remained stable throughout the multi day racing (Lombardi et al. 2014, Grasso et al. 2015). In summary, the impact of multiple exercise sessions on markers of bone turnover remains relatively unexplored and, to date, the interaction with pre-excise calcium intake is unknown.

Table 5: Acute Calcium Intake and Bone Turnover

Study	Population	Study type	Parameters/Methods	Findings/conclusions
Guillemant (Guillemant et al. 2004)	12 M elite triathletes	Randomised, placebo controlled, crossover trial	Exercise: 60 min 80% VO2max cycling Calcium: With and without 1000mg oral calcium load (mineral water, 1-hour pre and every 15 mins until 15 mins prior to the end of exercise) Markers: Pre, during, post, iCa, Phosphate, PTH, BAP, β-CTX-I measures	iCa was ↑ by calcium and maintained by placebo. by the Placebo showed ↑ β-CTX-I concentration and total amount relative to calcium group. Rise in PTH was partly suppressed by calcium load. BAP was not different between groups.
Barry (Barry et al. 2007)	20 M competitive road cyclists	Descriptive study	Exercise: 2 hours moderate intensity cycling (60-75% VT) Markers: Pre and post exercise PTH and iCa Dermal calcium loss using sweat patch collection	PTH ↑ in response to exercise There was no change in iCa when adjusted for HCT between pre, mid and post exercise. Neither iCa nor dermal Ca were significantly correlated with PTH.
Barry (Barry et al. 2011)	20 M competitive road cyclists	Double blind, randomised, placebo-controlled intervention	Exercise: 3x 35km cycling time trials Calcium: 1000mg calcium supplementation either 20 mins pre, every 15 mins during intervention or placebo at all time points. Markers: PTH, β-CTX-I, BAP, iCa measured pre and post exercise	iCa ↓ and β-CTX-I ↑ in response to exercise Ca supplementation before exercise attenuates disruption of PTH but did not affect β-CTX-I, BAP or iCa.
Haakonssen (Haakonssen et al. 2015)	32 F well trained cyclists	Randomised, counterbalanced, crossover design.	Exercise: 90 min cycling trial x 2; Calcium: dietary calcium 2 hours pre-exercise or placebo (1352mg v 46 mg) Markers: iCa, β-CTX-I, CTX-II, PTH, P1NP measured pre and immediately, 40 min, 100min and 190 min post exercise, dermal calcium loss using sweat patch collection	Calcium pre-exercise attenuated increases in PTH and β-CTX-I but not CTX II or P1NP. No correlation was found between dermal calcium loss and markers of bone turnover.

Study	Population	Study type	Parameters/Methods	Findings/conclusions
Sherk (Sherk et al. 2017)	51 M	Randomised, double blind crossover trial	Exercise: 2x 35 km cycling time trial Calcium: oral 1000mg calcium or placebo 30 min prior to exercise start Markers: iCa, PTH, β-CTX-I pre, immediately and 30 mins post	Calcium supplement attenuated the decline in iCa and increase in PTH (ns) but did not change β-CTX-I. The authors suggest the supplement may need to be taken earlier prior to exercise.
Kohrt (Kohrt et al. 2018)	11 M cyclists	Counterbalanced, crossover design, calcium trial first.	Exercise: 60 min cycling x 2 Calcium: saline or calcium infusion (iCa clamp) Markers: iCa, tCa, PTH, β-CTX-I, P1NP at 30 mins pre, immediately prior, every 15 mins during and for the 4-hour post exercise period.	iCa was ↓ in the first 15 minutes of exercise under both conditions but overall was ↓ with saline and maintained with calcium. PTH and β-CTX-I were ↑ at the end of exercise on the saline trial and markedly attenuated by calcium. PTH returned to baseline 1 hour post exercise, β-CTX-I remained elevated 4 hours post. Authors suggest exercise induced increase in PTH is generated to protect iCa and is catabolic to bone. The cause of the early drop in iCa is unknown.
Kohrt (Kohrt et al. 2019)	12 M 13 W cyclists	Randomised, counterbalanced, crossover design.	Exercise: 60 min cycling at 75% peak aerobic power x 2 Conditions: warm 26^{o}C or cool 18^{o}C Markers: iCa, tCa, PTH, β-CTX-I collected before during and up to 2 hours post exercise, dermal calcium loss through sweat patch collection	Sweat volumes were 50% higher for the warm conditions but there were no differences between conditions for iCa, PTH or β-CTX-I. Marked iCa decline occurred before substantial dermal calcium losses.

Study	Population	Study type	Parameters/Methods	Findings/conclusions
Wherry (Wherry et al. 2019)	12 older adults 7 M 5 W	Counterbalanced, crossover design.	Exercise: 60 min walking x 2 Conditions: warm 26°C or cool 18°C Markers: iCa, tCa, PTH, β-CTX-I sampled every 15 mins up to 60 mins post exercise.	Cool conditions undertaken first to allow screening for exercise tolerance before adding heat. Sweat volumes were low and not different between cool and warm conditions. The increase in PTH and β-CTX-I despite low dermal losses suggest that dermal losses are not the major trigger for responses.
Wherry (Wherry et al. 2021a)	12 older adults 6 M 6 W	Counterbalanced, crossover design, calcium trial first.	Exercise: 60 min brisk walking x 2 Calcium: saline or calcium infusion (iCa clamp) Markers: iCa, tCa, PTH, β-CTX-I, P1NP sampled before and every 15 mins during and periodically up to 4 hours post exercise.	iCa was ↓ with saline and maintained with calcium. Increases to β-CTX-I and PTH were attenuated in the calcium condition. This confirms previous findings and extends these to older individuals and with lower intensity exercise.
Shea (Shea et al. 2014)	10 W post-menopausal	Randomised, double blind crossover trial	Exp 1 60 min vigorous walking x 2; calcium fortified or control beverage before and every 15 mins during exercise. Exp 2 60 min vigorous walking x 2; calcium fortified or control beverage 15 mins pre. Markers: iCa, PTH, β-CTX-I before and after exercise	Exp 1- iCa ↓ in control but not calcium, PTH ↑ in both but attenuated with calcium, β-CTX-I only ↑ with control condition. Exp 2- iCa ↓ in both control and calcium but was attenuated in the calcium and there was no difference between groups for β-CTX-I or PTH. The authors conclude the timing of calcium intake is important.

3. Methodology and Design

Study 1: Nutrition Factors Associated with Rib Stress Injury History in Elite Rowers

Participants

This study was cross-sectional in nature, undertaken in an international level Australian rowing population (n=133) from senior (international level: n = 115; male = 67, female = 48) and under-23 levels (n = 18; male = 10, female = 8) of competition with recruitment between 2011 and 2015. All current national level athletes and those recently retired within the study period were invited to participate in the study, with a response rate of 85%. This study was approved by the Australian Institute of Sport Ethics Committee (Approval Number 20130208R3) and all participants provided written informed consent. Additional consent was obtained for inclusion in the case series. The rowers were provided with feedback of their individual results and outcomes disseminated to Rowing Australia athletes and staff including potential practical applications.

Study Procedure

Participants completed a standardised online questionnaire (www.surveymonkey.com) containing background information such as event discipline (sweep or scull), weight category (heavy or lightweight), training age (defined as age at study participation minus age of commencing rowing), sex, training habits, history of rib stress injury, diet restriction and menstrual background. The questionnaire was reviewed by the medical support team for appropriateness of content. Body composition and BMD measures were collected and assessed by a trained technician via dual-energy X-ray absorptiometry (DXA) at the Australian Institute of Sport using a GE Lunar Prodigy, Encore v13.6, according to standardised presentation (overnight fasted, rested and wearing minimal clothing) and positioning protocols previously as outlined by Nana et al, 2015 (Nana et al. 2015) namely with the participant centrally aligned in a standard position with custom made positioning aids. Anterior posterior spine (L1-L4) and proximal femur scans were used to calculate bone mineral density in g/cm^2 and classified using age-matched and sex specific Z-score index (Geelong/Lunar). Rib BMD was identified using the DXA machine software automated breakdown for whole body composition report for ribs. This consists of the trunk segment including all ribs, excluding vertebral

bodies, and spine with and separated from the arms before the acromion (Figure 6); Z-scores were not available for this measure.

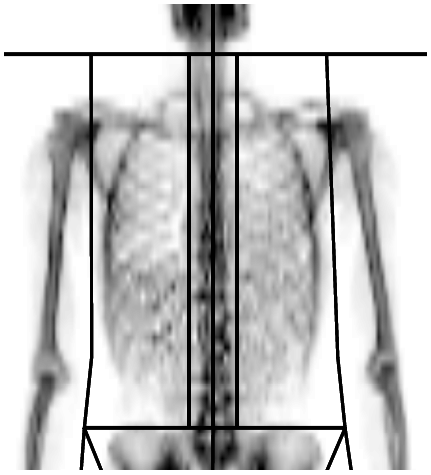


Figure 6 Region of Interest Used for Rib BMD

Upper and lower body lean mass was defined by the DXA software regions of interest as trunk plus arms, and legs respectively. Low body fat was defined as 5% or less (Friedl et al. 1994) for men and <12% for women (Klungland Torstveit et al. 2012). Low body mass was defined as BMI <17.5 kg/m^2 and osteopenia as Z < -1 (De Souza et al. 2017, Fredericson et al. 2021). Anthropometric measures included arm span, sitting height, acromiale-radiale length, radiale stylion length, trochanterion-tibiale laterale length plus tibiale laterale height, biacromial breadth according to standardised measurement protocols by the International Society for the Advancement of Kinanthropometry (Esparza-Ros et al. 2019).

The following three levels/degrees of diet restriction were defined by self-reported questionnaire responses: no diet restriction ("no, I eat as much as I like/need most of the time"), minor diet restriction ("yes, I watch what I eat but can still eat fairly freely"), and considerable diet restriction ("yes, I am actively trying to lose weight/body fat to meet a target" and "yes, I restrict what I eat most of the time to manage my body weight/composition"). Habitual calcium intake was estimated using the validated Short Calcium Questionnaire SCQ2002 (Sebring et al. 2007), administered by a qualified dietitian in person or over the phone. Calcium-equivalent Australian foods were substituted for the American-based food composition data in this questionnaire. Adequacy of intake was set at 1,000 mg, in keeping with the Recommended Dietary Intake for Australians 19-50 years (National Health and Medical Research Council 2006).

A sub-group of participants (n= 68) had assessment of vitamin D (25-hydroxyvitamin D (25(OH)D) and vitamin K (phylloquinone) status and triglyceride concentrations. These were measured on fasting blood samples, collected by trained phlebotomists, between the months of July and October (winter/spring). Vitamin D insufficiency was defined: <80 nmol/L and deficiency: <50nmol/L (Ogan et al. 2013). The vitamin D and K assays were undertaken by a commercial laboratory (Laverty

Laboratory, North Ryde, Sydney, Australia). 25-hydroxyvitamin D (25(OH)D) was measured using the Diasorin Siemens chemiluminescent assay as previously described (Farrell et al. 2012) and vitamin K was extracted from serum by ethanolic protein precipitation followed by solid phase extraction on SPE cartridges. An isopropanol eluate was evaporated to dryness and the residue containing the vitamin K was separated by reversed phase HPLC. Post-column reduction with platinum enabled measurement with a fluorescence detector. Triglyceride concentrations were assessed using an enzymatic colorimetric assay (Integra 400+, Roche Diagnostics, Basel, Switzerland).

Participants were asked to record the month and year of any rib stress injuries sustained during their rowing career. Retrospective analysis of clinical records kept by Rowing Australia were used to confirm all occurrences, and to confirm that all rib stress fractures were accounted for. Further information on these records can be found in previous publication of this cohort (Harris et al. 2020, Trease et al. 2020). If the case of a discrepancy, both the athlete and clinical staff were contacted to confirm the case and final diagnosis. For the purpose of analysis, rib-related injuries were grouped sequentially as chest wall pain (early signs of injury such as pain requiring more than 24 hours off water, according to the injury protocol (Hooper et al. 2011) but without imaging being undertaken) or rib stress injury (incorporating rib stress reaction and rib stress fracture confirmed by imaging). Data were combined into a single de-identified database for analysis.

Statistical Methodology

While cross-sectional in design, we analysed the data as a case-control. A case was defined for analysis as a participant who reported a rib injury prior to the data collection. A control was a participant who remained rib injury free prior to and during the career to date of assessment. Injury history was analysed in a binary classification with athletes who reported more than one injury (n=6) separately described as a case series, in addition to being included in the main analysis.

One-way ANOVAs were performed on characteristics between injured and uninjured rowers. The effect size (ES) measures were defined by Cohen's d statistic (Cohen 2013). Multiple linear regressions were used to assess the relationship between a set of explanatory variables (sex, age, training age, weight category, diet restriction, history of rib stress, vitamin D, vitamin K, calcium) and response variables (rib BMD, spine BMD and Z-score, and femur BMD and Z-score). To identify factors associated with RSI, multiple logistic regression models were developed. Explanatory variables included age, sex, rib, spine and femur BMD, weight category, diet restriction, and body fat percentage. In addition, for female rowers, models included current menstrual status and menarche.

Study 2 Screening for Low Energy Availability in Male Athletes: Attempted Validation of LEAM-Q

A total of 405 male athletes were recruited in a multi-center study, through the Australian Institute of Sport, the Norwegian Olympic and Paralympic Committee and Confederation of Sports, the University of Copenhagen and the University of Agder. Inclusion criteria were male elite and sub-elite athletes, 18-50 years old with an absence of thyroid or metabolic disease with 310 meeting criteria and completing all aspects adequately for inclusion (88%). All subjects received information regarding the background of the study, test procedures and signed an informed consent document. Ethics approval was granted by the Australian Institute of Sport Ethics Committee, the Capital Region of Denmark, the University of Agder's Faculty Ethics Committee, the Norwegian Regional Committees for Medical and Health Research Ethics and the Norwegian Centre for Research Data (NSD). The questionnaire was created using content from the LEAF-Q (Melin et al. 2014), ADAM-Q (Mohamed et al. 2010), literature review and expert consultation for content validity. Each question was scored on a Likert-type ordinal or nominal scales with a higher score indicating a greater likelihood of LEA. The validation was assessed in a two-step process, first for internal consistency and reliability (n=53) and secondly, in a separate participant group, to verify the self-reported symptoms from the questionnaire against measured clinical markers associated with LEA (n=352). The questionnaire initially included 33 items covering dizziness, gastrointestinal function, injury and illness and well-being and recovery. The questionnaire was revised part way through collection and increased to 42 items, with additional questions on dizziness, wellbeing and recovery, sleep and sex drive. Sex drive questions were initially not included in order to make the questionnaire more comfortable to administer and discuss across a range of male athlete populations however on review of initial results was added to improve sensitivity of the questionnaire. Both versions of the questionnaire included questions to provide demographic and athletic status information. Supplement 1 shows the initial version of the questionnaire prior to analysis with questions added during the revision highlighted in red. Supplement 2 shows the final questionnaire and associated scoring key with the sex drive being the sole section retained.

Clinical Verification of Self-Reported Symptoms

The LEAM-Q was completed on-line or on paper by 352 participants with 42 ultimately removed due to missing key data leaving 310 participants for analysis (183 in version 1, 127 in version 2). For the assessment of clinical markers, the subjects met at campus between 5 and 9 a.m. in a rested fasted state, with no fluid intake or prior physical activity on the morning of the assessment. Body weight was measured to the nearest 100g and height to the nearest millimeter using calibrated instruments at the different centers. Body composition was assessed in a resting supine position using standardized positioning as previously described (Nana et al. 2016) using Dual-energy X-ray absorptiometry (DXA)

in the total body and site-specific modes and on a narrow fan-beam DXA scanner (GE-Lunar Prodigy or iDXA, using GE enCORE analysis software version 15.0 or 16.2, Madison, WI, USA according to testing location) following appropriate machine calibration and in keeping with best practice guidelines (Nana et al. 2015). Bone mineral density (BMD) was assessed for proximal femur and anterior posterior lumbar spine (L1-L4). For the Scandinavian cohorts the combined NHANES/Lunar reference database was used and for the Australian cohort the combined Lunar/Geelong as deemed most appropriate for the respective populations. Low BMD was defined as BMD Z-score <-1 at any measured site (Table 6)(Mountjoy et al. 2018, Fredericson et al. 2021).

Resting Metabolic Rate (RMR) was measured either by metabolic cart (Oxycon Pro or Vyntus CPX, Jaeger GmbH, Hoechberg, Germany) or the first principles method (Haugen et al. 2007) depending on the testing location. All measures were taken in a warm, quiet, and dimly lit room. For the metabolic cart method, a ventilated canopy hood system was used to assess RMR, with systems being calibrated before each test according to standards, and alcohol calibration weekly. Subjects rested for 15 minutes prior to collection. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were assessed over a 30-minute period and converted to kJ/min based on the Weir equation (Weir 1949). The last 20 minutes of measurement were used to assess RMR using the protocol defined by Compher et al. (Compher et al. 2006). The first principles method replicated the processes as previously described (Bone et al. 2018). To calculate the RMR_{ratio}, the Cunningham (1980) equation (Cunningham 1980) was used to calculate the predicted RMR of each subject: 500 + (22 × LBM [kg]). REE was also calculated relative to FFM as determined by DXA (kJ/kg FFM). As systematic differences were noted between the first principles and metabolic cart measurements the lowest quartile of each method was used to indicate a 'low RMR' finding (Table 6).

Blood pressure (BP) was obtained in a resting supine position using an electronic sphygmomanometer (Microlife BP A100, Widnau, Switzerland or HEM7320, Omron Healthcare, JA Davey Pty Ltd, Melbourne, Australia). The monitor was secured around the participant's left upper arm, and automatically provided a reading of systolic and diastolic blood pressure, and resting heart rate.

Blood samples were collected after within 30 minutes of completion of the RMR measurement, obtained via venipuncture from an antecubital forearm vein by a qualified phlebotomist. This ensured samples were fasted, rested and collected at a similar time of day for all subjects (Hackney et al. 2008). For the Scandinavian cohort blood was clotted at room temperature for 30 minutes before being centrifuged at 1300 g for 10 minutes. Serum was transferred into tubes and stored at -80°C until analyses. The serum from Kristiansand was analyzed at St. Olavs Hospital (Trondheim, Norway) and serum from Oslo was analyzed at Fürst medical laboratory (Oslo, Norway), for its content of glucose, insulin, cortisol, total TES, free triiodothyonine (T_3), and IGF-1. For the Australian cohort a single venous blood sample (2 x 8.5 ml serum separator tube) was used for the assessment of fasting IGF-1, cortisol, lipids, insulin, TES and T_3 for analysis by chemiluminescent immunoassay through a commercial

laboratory (Laverty Pathology, Bruce, ACT, Australia). IGF-1 was assayed using the DiaSorin Liason® XL (DiaSorin Diagnostics, Sallugia, Italy), whilst cortisol, TES and T_3 were assayed using the Siemens ADVIA Centaur XP (Siemens Healthcare Diagnostics Ltd, NY, USA) as per manufacturer's recommendations. Fasting blood glucose levels were assessed via fingertip capillary sample using a portable meter and test strip (Accu-Chek® Performa, Roche Diagnostics, Castle Hill, Australia). Free TES was calculated from total TES, sex hormone binding globulin and albumin or where unavailable 43 g/L according to the method by Vermeulen et al (Vermeulen et al. 1999). As the blood analyses were conducted at different laboratories a 'low' finding was determined using the lowest quartile of the reference range for the laboratory at which the measure was taken (Table 6).

Internal Consistency and Reliability

To assess items performance and estimate reliability, a test-retest were performed. Forty-two participants from Australia, Norway and Sweden received the LEAM-Q in either English, Norwegian or Swedish, as appropriate. The participants were asked to complete the questionnaire twice, 14-days apart. After the re-test, researchers asked the participants to identify any concerns they had with the items including ease of understanding, relevance, and the appropriateness of the possible answers. Questionnaires were identified by subject number only and were collected either on paper or secure electronic format; Microsoft Forms or SurveyXact, (8200 Aarhus, Denmark).

Statistics

To assess items performance and estimate reliability, the intraclass correlation coefficient (ICC) was used to calculate the difference between the test and the retest score using a Two-Way mixed random effects model.

The association between clinical outcomes and LEAM-Q variables were assessed including all subjects (n=310) using multivariate linear or logistic regression models for all combinations of clinical outcomes (as responses) and screening variables from LEAM-Q (as predictors), including adjustment for age, BMI, elite athlete (yes/no), center (if there were data from multiple centers). In addition to the standard questionnaire scoring, a separate score was conducted for symptoms included in the EHMC (Hackney et al. 2020). This was assessed as a score for the libido questions "In general I would rate my sex drive as", "Morning erections over the last month" and "How many morning erections compared to normal" in combination with the items "I feel tired from work or school", "I feel lethargic", "I feel strong and making good progress with my strength training", "I feel very energetic in general", I feel invigorated for training sessions and ready to perform well", "I feel happy and on top of my life outside of sport". Low libido was also categorized by using sex drive scores equal or greater than 2 on "Sex drive in general" or equal or greater than 2 on "The number of morning erections" and equal or greater than 1 for "Morning erections compared to normal" to represent reproductive dysfunction. For LEAM-Q variables with significant association to one or more clinical outcomes ($p<0.05$) optimal sensitivity was estimated from separate ROC curve analyses using Youden's index to determine optimality. At least

60% sensitivity was required to identify potentially useful screening variables which were retained. For clinical variables classed as “high” or “low” this represented the test locations highest or lowest 25% percent of results, respectively. Data were analyzed using R (R Core Team 2020. R Foundation for Statistical Computing, Vienna, Austria) with the following extension packages Hmisc (Harrell 2020) and pROC (Robin et al. 2011).

After excluding 45 subjects on the basis of missing at least 3 clinical markers, including at least one primary clinical marker the remaining subjects (n=265), were categorized as LEA-cases or controls by using the system outlined in Table 6. A two-sample t-test were used to analyze differences in the retained LEAM-Q variables between cases and controls.

Table 6 Definition of Clinical Indicators of LEA

Primary Indicators	Secondary indicators
1. **Low T_3:** lowest quartile (<3.5pmmol/l). 2. **Low total or free testosterone:** Lowest (<16 nmol/L, <333 pmol/l respectively). 3. **Low BMD:** Z-score <-1 for either AP spine or proximal femur (Nattiv et al. 2007, Mountjoy et al. 2018, Fredericson et al. 2021). 4. **Low body weight:** Body Mass Index (BMI) <18.5 kg/m^2 (De Souza et al. 2014, Fredericson et al. 2021).	1. **Low RMR_{ratio}** lowest quartile for the testing method (<1.11 for first principles and <0.88 for metabolic cart measures). 2. **Hypotension:** <90mmHg systolic and/or diastolic <60mmHg (Melin et al. 2014). 3. **Low body fat:** <5% as measured by DXA (Friedl et al. 1994). 4. **Low IGF-1:** lowest quartile of the age dependent reference range at the testing site. 5. **High LDL cholesterol** (>3mmol/l) (Melin et al. 2014). 6. **High cortisol** (>550nmol/l) or **cortisol (nmol/l) insulin ratio (pmol/l)** (>26.6).

Subjects were categorized as LEA if they had two or more primary indicators or three or more indicators overall

Study 3 The Impact of Acute Calcium Intake on Bone Markers During a Training Day In Elite Rowers

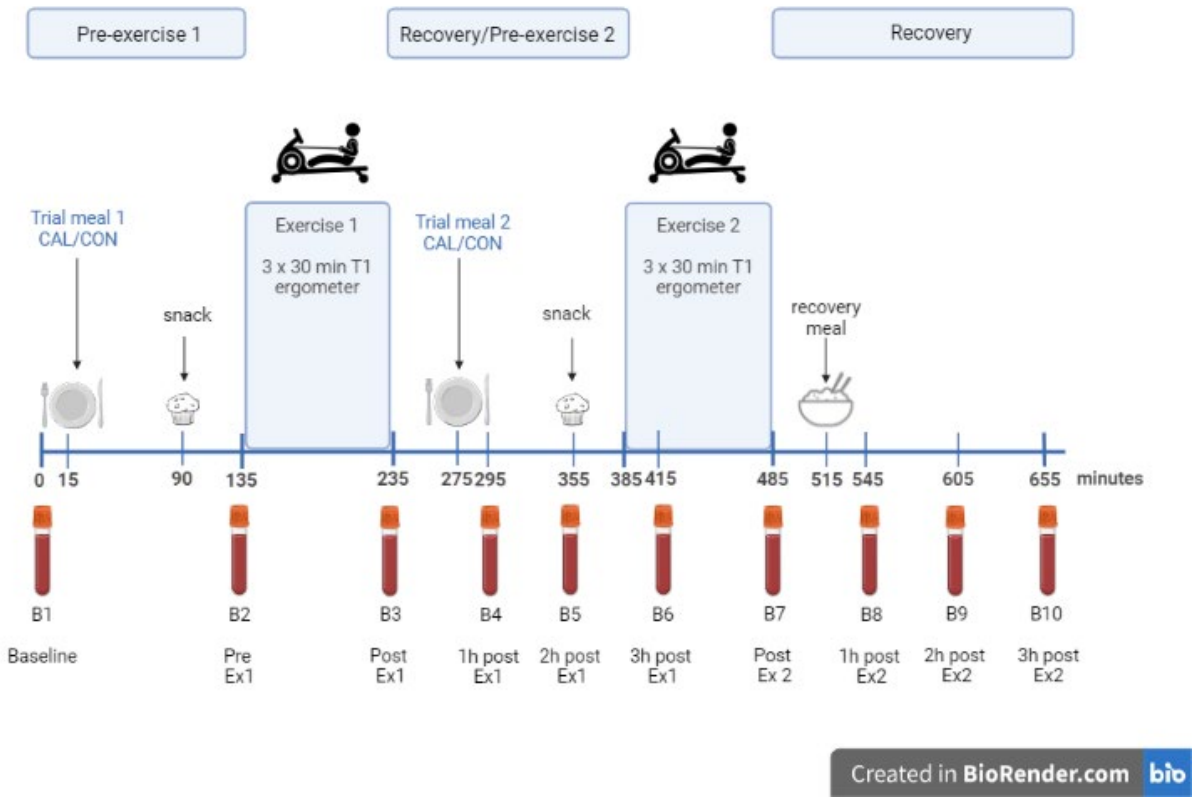
Participants

Eighteen elite male rowers from the Rowing Australia National Training Centre, in preparation for potential Olympic representation, were recruited for this study and a parallel study investigating iron and hepcidin responses (Fensham et al. 2021b). One participant with newly identified food intolerances was excluded due to his inability to complete one of the dietary arms, while another was unable to complete the required training load due to recent illness. The final 16 participants are characterized in Table 1. Written informed consent was obtained from each athlete prior to study commencement. Ethics approval was obtained from the Australian Institute of Sport Ethics Committee (ref: 20200905).

Experimental Overview

In a randomized, crossover design, athletes completed two trials, one week apart, involving either a high (CAL) or low calcium (CON) dietary intervention (see Figure 7).

Figure 7 Experimental Overview



While it was not possible to make the intervention meals identical, they were matched as closely as possible and neither the athletes nor research personnel involved in data collection and entry were aware of allocation of the treatments. Subjects were tested on the same day of the week, with the weekly training prescription duplicated for both trials 1 and 2. On trial mornings, athletes arrived at the laboratory (0600-0700) in an overnight fasted, rested state, and a blood sample was collected from a cannula placed in a forearm vein (t = 0 min). Athletes then consumed either a low (<10 mg) or high calcium (1000 mg) standardized breakfast (t = 15 min). After 115 min, a pre-exercise whole blood sample was drawn and 5 min later (t = 135 min) the first exercise session (EX1) commenced. Each exercise session comprised of three 30-min sets on a rowing ergometer, separated by 5 min and was designed to replicate a typical exercise session on water. Immediately post-EX1, blood was sampled, and at t = 265 min (30 min post EX1 and 120 min prior to EX2) a second low or high calcium meal was consumed according to group allocation. Blood samples were drawn 1 and 2 h post-EX1 (t = 295 and 355 min). At t = 385 min, a repetition of the earlier session was undertaken (EX2), noting a recovery period of 150 min between exercise bouts. Blood was collected at the break between the first and second sets of EX2 (t = 415 min, equating to 3 h post-EX1). Blood was collected on completion of EX2 (t = 485 min), prior to the consumption of a recovery meal, with further samples at 1, 2, and 3 h post-EX2 (t = 545, 605, and 665 min).

Exercise sessions were completed on a rowing ergometer (Concept 2, Morrisville, Vermont, USA), drag factor 130. Several rowers (n=5), who had a current injury or injury risk undertook one of their 30 min exercise sets, in each of Ex1 and Ex2, on a Wattbike Pro cycling ergometer (Wattbike Ltd., Nottingham, UK), replicating real-world practice. Meanwhile, another participant undertook all trials on the Wattbike. Session intensity was individually prescribed at 90-100% of the power previously identified from an incremental test as the point at which capillary blood lactate reached 2 mmol/L. Mean power was recorded for each effort, as was HR (beats per min; Wahoo Tickr X, Wahoo Fitness, Atlanta, USA) and subjective rating of perceived exertion (RPE) according to the Modified Borg Scale (6-20)(Borg 1970).

Bone Mineral Density and Body Composition

Body composition and bone mineral density (anterior posterior (AP) spine (L1-L4), proximal femur) were measured by dual x-ray absorptiometry (DXA) in the morning, fasted and rested, according to methods described previously (Nana et al. 2016) (GE Healthcare, Lunar iDXA, Encore v16.2). As the subjects were too tall for the scanning bed, body composition was assessed, summing regions of

interest for the trunk, arms and legs to provide a total body less head composition as per best practice (Nana et al. 2012).

Dietary Standardisation:

Subjects followed a standardized diet for the 24 h prior to each of the two experimental trial days, individualized by a sports dietitian according to body weight, habitual diet and intolerances and following recommendations by Jeacocke and Burke (2010). This diet was provided to subjects as pre-packaged food items with verbal and written instruction to direct intake. The nutrient prescription for these diets was 256 kJ/kg body mass (BM); energy; 8 g/kg carbohydrates (CHO); 2-3 g/kg protein and 35% of energy from fat. Substitutions were made as required for gluten free (n = 1), lactose-intolerant (n = 1) and other food sensitivities (n = 1) while retaining the macronutrient targets. Abstinence from alcohol intake and habitual*/ad libitum* intake of caffeine and fluid intake were maintained, with recording of intake to allow replication for each trial. Subjects were permitted to consume additional foods according to hunger on the first week, with a checklist being provided to note any deviations which allowed replication during the second trial. These were checked on arrival for both trial mornings by the dietitian.

On the trial day food was prepared, served, and consumed from the on-site kitchen according to the trial schedule. Subjects consumed the same meals and snacks for each trial day except for the targeted pre-exercise intervention meals. The interventions consisted of a CAL (high calcium, 1000 mg) or CON (low calcium, <10 mg calcium) menu of Bircher muesli and a toasted sandwich (Table 7).

Table 7: Intervention Meal

Intervention	**Meal components**	**Nutrient breakdown**
CON (low calcium meal)	**Low Calcium Bircher Muesli:** Nutty Bruce Roasted Almond & Oat Milk (200 mL) Kingland Dairy Free Greek Style Yoghurt Mixed Berry (170 g) Carman's Bircher Muesli (90 g) **Low Calcium Toasted Sandwich:** Helga's Mixed Grain Bread (2 Slices) Ham (100 g) Butter (9.5 g) My Life Bio Cheese (40 g)	Energy 4819 kJ Protein 49 g Fat 50 g Carbohydrate 127 g Calcium 1 mg
CAL (high calcium meal)	**High Calcium Bircher Muesli:** Pauls High Calcium Milk (200 mL)	Energy 4819 kJ Protein 61g

	Siggis High Calcium Vanilla Yogurt Carmans Bircher Muesli (90 g) **High Calcium Toasted Sandwich:** Helga's Mixed Grain Bread (2 Slices) Ham (50 g) Butter (9.5 g) Bega Tasty Cheese (50 g)	(125 g)	Fat 52 g Carbohydrate 108 g Calcium 1,042 mg

For CAL, dairy foods were the primary contributors to the calcium target, and easily met this 1000 mg goal within portion sizes typically consumed in a breakfast meal in this population. For CON, a non-dairy yoghurt, non-fortified almond milk and vegan cheese were used as substitutes. In designing these menus, priority was given first to achieving calcium targets, then to matching carbohydrate and energy content of the meals with the protein and fat matched as closely as possible. At 75 min post-meal, all participants consumed a pre-exercise snack (muffin). During the exercise sessions water was provided *ad libitum* and recorded accordingly. Body mass was measured pre- and post-exercise to allow estimation of fluid losses through sweat (with adjustment for the volume of fluid consumed and any urine losses during the session). A CHO-rich gel (Science in Sport PLC, London, UK) or carbohydrate equivalent quantity of confectionary was consumed (~30 g CHO) during each 5 min break between exercise sets. To maintain real-life practice, without disturbing the study intervention, athletes were able to request more ("calcium-free" < 10 mg) food in the recovery period between exercise sessions in addition to the pre-exercise meal. Any additional foods consumed in the first trial were recorded and repeated in the second trial. The nutrient composition of all diets was calculated using a computerized dietary analysis package (Nutritics Ltd., Dublin, Ireland) by the same sports dietitian.

Thirty minutes after the completion of the first exercise session, the pre-exercise meal and snack were provided in the same sequence before the second exercise session. On completion of this session, all participants consumed a recovery meal (butter chicken or beef and black bean, rice, and vegetables) and snack (chocolate chip cookie). The quantity of this meal was self-selected in trial 1, recorded and replicated in trial 2.

Blood Analysis

During each trial, ten venous blood samples were collected into either 6- or 8-ml serum separator tubes (BD Vacutainer, Australia). Blood samples were taken at rest (fasted), pre-exercise (2 h post

breakfast), and immediately, 1 h, 2 h, and 3 h post each exercise session (Figure 1). Samples were left to clot for 30 min before being centrifuged at 1500 G for 10 min at 4°C. Serum was aliquoted into 1.5 ml cryotubes and frozen at -80°C until batch analysis was performed. PTH and vitamin D (25-hydroxyvitamin D (25(OH)D) concentrations were measured by chemiluminescent immunoassay (Access 2, Beckman Coulter, Brea, CA, USA), CV 4.5% and 6.5%, respectively. CTX concentrations were assessed by electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics, Basel, Switzerland); CV 4.2%. OC and ucOC concentrations were measured by an automated, non-competitive, chemiluminescent immunoassay performed on a Cobas e801 (Roche Diagnostics, Basel, Switzerland), CV 1.2% and 3.2%, respectively. Metabolic and reproductive hormones, free triiodothyronine (T_3) total (TES) and free TES (fTES), cortisol and insulin like growth factor -1 (IGF-1) were assessed by a commercial laboratory (Laverty Pathology, Bruce, ACT, Australia). IGF-1 was assayed using the DiaSorin Liason® XL (DiaSorin Diagnostics, Sallugia, Italy).

Capillary whole blood was used to determine ionized calcium, hemoglobin, and hematocrit (Hct) with the point-of-care i-STAT device (Abbott Point of Care Inc., Princeton, NJ, USA); CV 1.5% and sensitivity of 10%. Changes in bone turnover markers (BTM) were adjusted for hemoconcentration using methods described by van Beaumont et al. (Van Beaumont et al. 1972). Raw Hct readings were multiplied by a factor (0.96 x 0.91) to correct for plasma trapped between red blood cells and to convert venous Hct to whole-body Hct respectively. BTM concentrations were adjusted to the concentration expected (CE) based on fluid shifts alone (Equation 1), where changes in Hct from immediately pre-exercise (Hct1) to all post-exercise values (Hct2) were calculated (with C1 representing the initial concentration of the biomarker).

Equation 1: $CE = [Hct2(100- Hct1)]/[Hct1(100- Hct2)] \times C1$

Secondly, the unadjusted post-exercise biomarker concentrations (C2) were corrected for CE

(Equation 2), giving an Hct-corrected concentration (C2Hct).

Equation 2: $C2Hct = C2 – (CE - C1)$

All values for PTH, β-CTX-I, OC and iCa were adjusted for hemoconcentration and reported as both unadjusted and adjusted.

Statistical Analysis

Statistical analysis was performed using R Studio (R Core Team, 2021, v3.5.2). Linear mixed models were constructed to assess changes in hematocrit, iCa, PTH, β-CTX-I and OC, with fixed effects for Time and Condition, and Subject Identification and Week, used as random effects. Similar models were used to determined changes in training variables (power output, heart rate and RPE). Sweat loss was included as a covariate in the analysis of β-CTX-I to rule out any association with dermal calcium losses.

Visual inspections of residual plots were used to assess homoscedasticity and normality. Significant deviations were noted for PTH and β-CTX-I and, thus, data was log-transformed for analysis. Finally, the relationship between BMD (AP spine, proximal femur, and Z-scores) and β-CTX-I (pre-exercise concentrations, percentage change at 1 h post-exercise from pre-exercise for EX1 and EX2) was assessed via Pearson's correlations. Pre-trial and trial day dietary intake were compared with paired sample t-tests. Significance was set at $p<0.05$.

4. Nutrition Factors Associated with Rib Stress Injury History in Elite Rowers

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Rib Stress Fracture Research Project Demographic Questionnaire

***1. What is your date of birth?**

DD MM YYYY

DD/MM/YYYY / /

***2. Do you compete as a lightweight or heavyweight?**

- ◯ Lightweight
- ◯ Heavyweight

***3. Do you currently row Sweep or Scull?**

- ◯ Sweep (bow side)
- ◯ Sweep (stroke side)
- ◯ Scull

***4. Please indicate below how many seasons you have trained/competed in a boat class other than the one elected in question 3**

***5. What is the primary boat you have been selected for?**

- ◯ Single
- ◯ Double
- ◯ Quad
- ◯ Pair
- ◯ Four
- ◯ Eight

***6. How old were you when you started rowing?**

***7. How many years have you competed on the senior national team?**

Rib Stress Fracture Research Project Demographic Questionnaire

***8. How many times did you compete in the U23 national team?**

- ◯ never
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5+

***9. How many years have you competed in the junior national team?**

- ◯ never
- ◯ 1
- ◯ 2
- ◯ 3+

***10. Outside of ergometer and resistance training sessions, what is your main form of cross training?**

- ◯ Running
- ◯ Cycling
- ◯ A combination of both
- ◯ None of the above (please specify)

***11. In a normal training block, how many sessions a week would you be most likely to complete of the following training types?**

	Number of sessions
Stationary Ergometer	
Ergometer on sliders	
On water rowing	

Other (please specify)

Rib Stress Fracture Research Project Demographic Questionnaire

*12. In a typical week how many full days off (no training) would you usually have?

	Number of full days off
Pre-season (October to December)	
Domestic Season (January to April)	
International preparation (May to August)	

Other (please specify)

*13. In a typical week how many hours a week would you be most likely to train?

	Hours per week training
Pre-season (October to December)	
Domestic Season (January to April)	
International preparation (May to August)	

Other (please specify)

*14. Do you currently diet or restrict your food intake in order to make weight or achieve your body composition goals for rowing? Choose the answer that best suits your situation

- ◯ No, I eat as much as I like/need most of the time
- ◯ Yes, I watch what I eat but I can still eat fairly freely
- ◯ Yes, I am actively trying to lose weight/body fat to achieve a target
- ◯ Yes, I restrict what I eat most of the time to manage my body weight/composition

Other (please specify)

*15. Have you ever had a rib stress injury

- ◯ Yes
- ◯ No

Rib Stress Fracture Research Project Demographic Questionnaire

***16. Please indicate below your history of rib injury. If you have had no rib stress injuries, just answer 0 in the first column.**

	How many have you experienced?	On which side of the body did they occur?	What month did they occur?	What year did they occur?	Were you rowing sweep or scull at the time?
I have had rib/chest wall pain					
I have had a rib stress reaction					
I have had rib stress fracture/s					

Other (please add any other information you think is relevant here)

Rib Stress Fracture Research Project Demographic Questionnaire

Page 2

*17. Please indicate below your history with low back pain

- [ ] I have never missed training due to low back pain
- [ ] I have missed minimal training due to low back pain (one week or less for a given incident)
- [ ] I have missed a moderate amount of training due to low back pain (periods of more than one week)
- [ ] I require ongoing modification to training due to low back pain

*18. Gender

- ◯ Male
- ◯ Female

Rib Stress Fracture Research Project Demographic Questionnaire

For females only

***19. What age were you when you first got your period (menstruated)?**

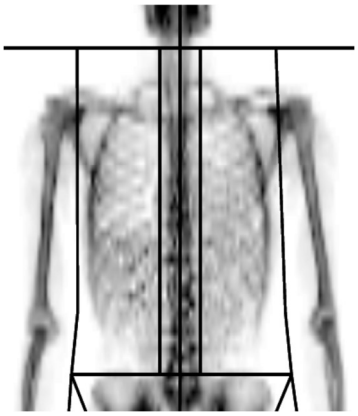
***20. Please choose the response that best describes how regular your period currently is**

- ◯ I use the oral contraceptive pill or another form of hormonal contraception but my periods were regular prior to starting this
- ◯ I am using the oral contraceptive pill or another form of hormonal contraception due to irregular or absent periods
- ◯ I am not on the pill and my periods are irregular
- ◯ I am not on the pill and my periods are regular
- ◯ I have never had a period/menstruated
- ◯ Other (please specify)

***21. Please choose the response that best describes how regular your period has been since you first got it?**

- ◯ My periods have always been regular
- ◯ I have had months where I have missed my period (not due to use of the oral contraceptive pill)
- ◯ I have had one episode of more than six months without a period
- ◯ I have had more than one episode of more than six months without a period
- ◯ Other (please specify)

Supplement 2



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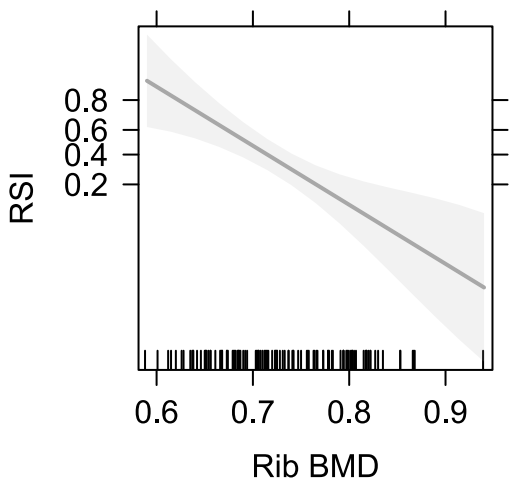

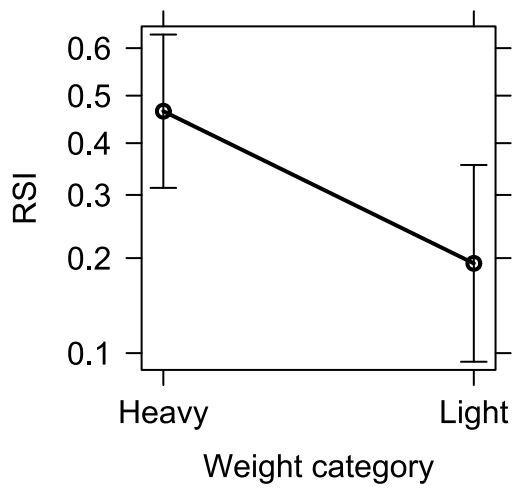

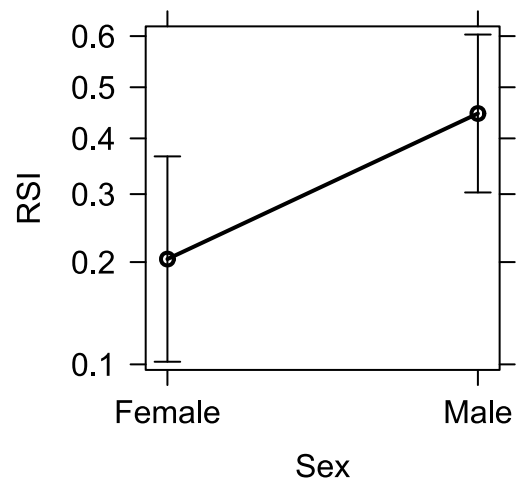

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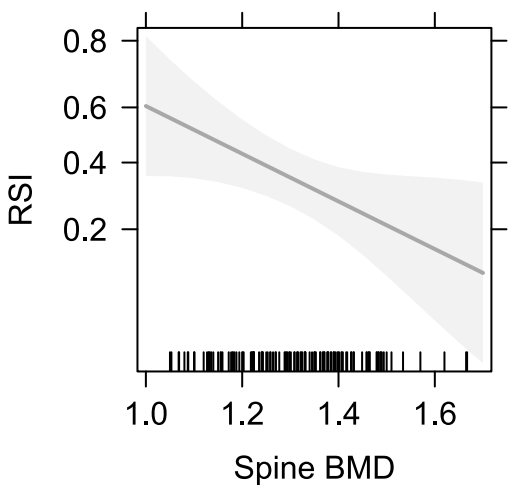

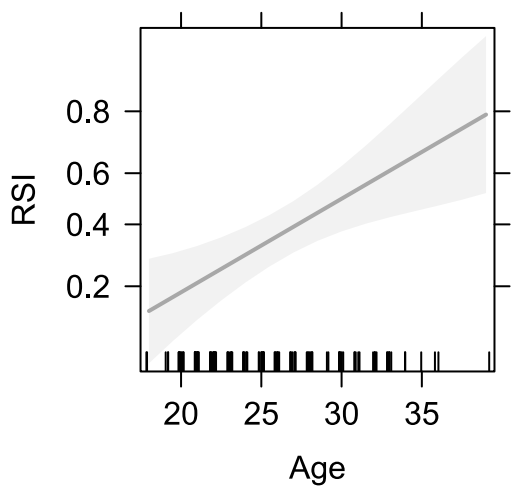

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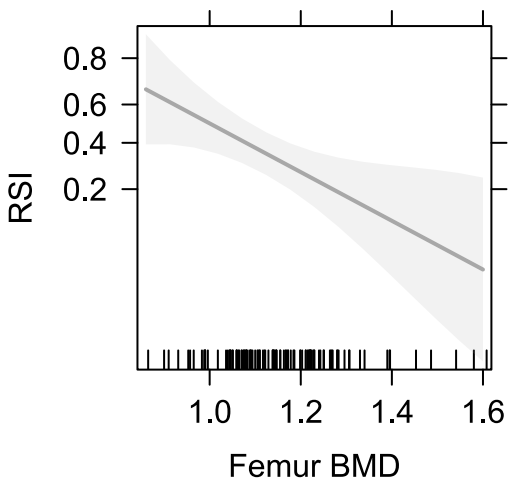

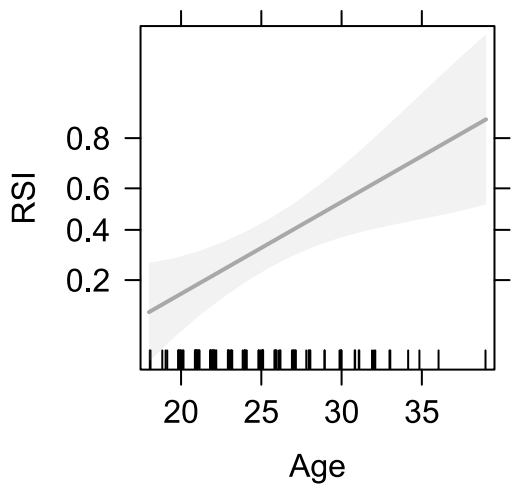

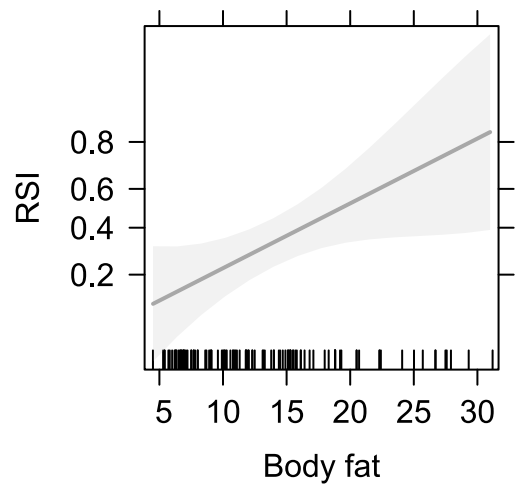

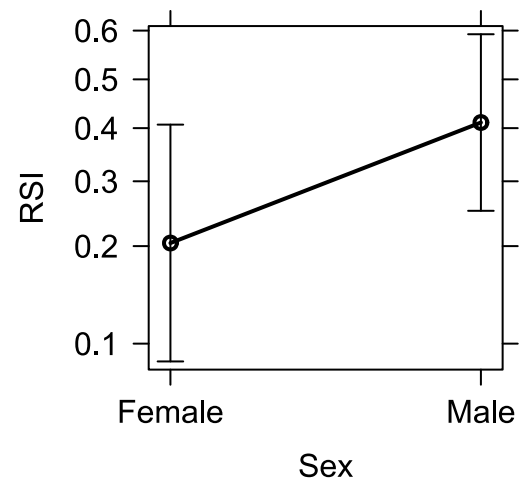

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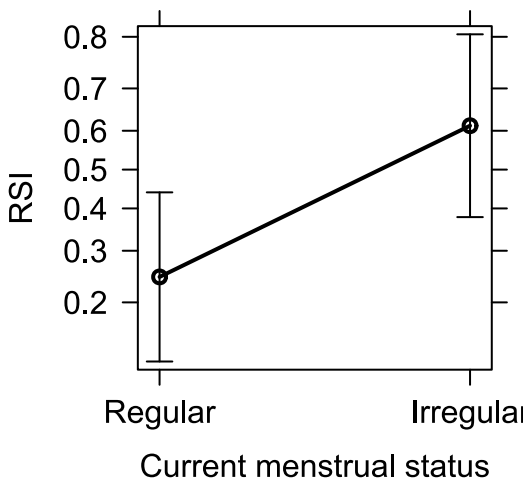

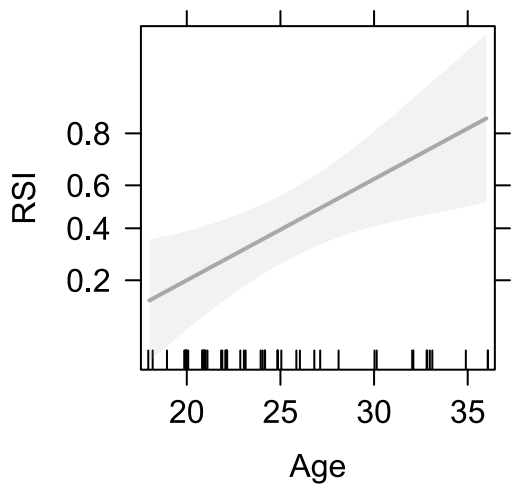

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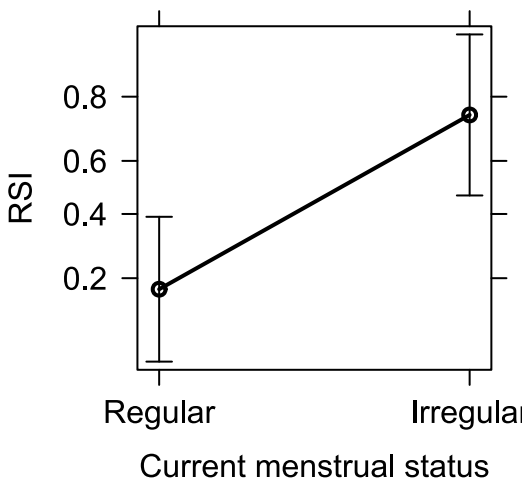

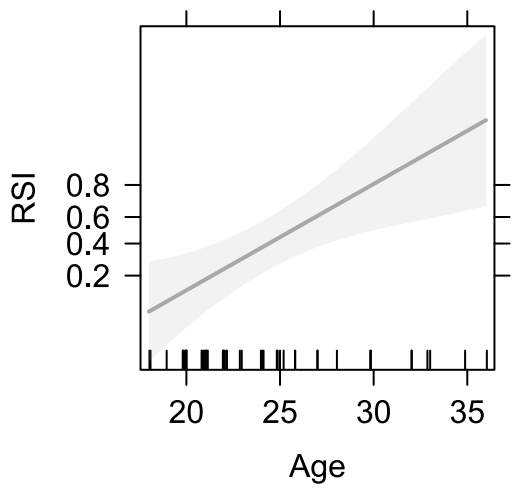

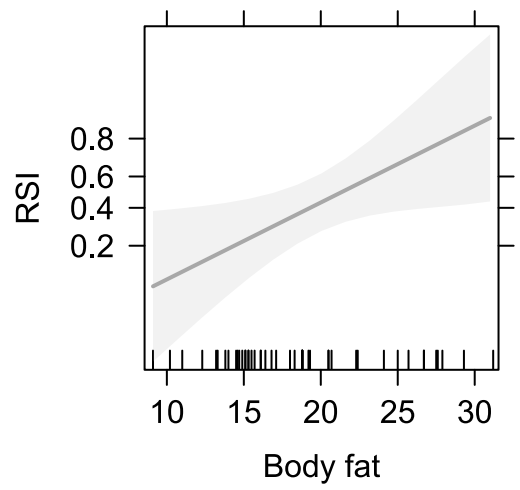

Supplement 3

Supplementary Table 1 Multiple logistic regression models evaluating factors associated with RSI in rowers

(a) Rib BMD

	RSI Model 1			**RSI Model 2**		
Coefficient	*Odds Ratios*	*CI (95%)*	*P*	*Odds Ratios*	*CI (95%)*	*P*
Age				1.19	1.07 – 1.35	**0.003**
Body fat				1.15	1.03 – 1.30	**0.019**
Female *(Reference)*						
Male	3.17	1.04 – 10.50	**0.049**	5.44	1.18 – 28.43	**0.036**
Rib BMD (scaled)*	0.14	0.04 – 0.41	**0.001**	0.25	0.10 – 0.56	**0.001**
Heavyweight *(Reference)*						
Lightweight	0.27	0.08-0.88	**0.037**			
Observations	111			111		
AIC	136.359			130.658		

(b) Spine BMD

	RSI Model 3			**RSI Model 4**		
Coefficient	*Odds Ratios*	*CI (95%)*	*P*	*Odds Ratios*	*CI (95%)*	*P*
Age	1.17	1.07 – 1.29	**0.001**	1.21	1.08 – 1.38	**0.002**
Body fat				1.12	1.01 – 1.27	**0.035**
Female (*Reference*)						
Male	0.69	0.30 – 1.55	0.368	2.05	0.53 – 8.73	0.310
Spine BMD (scaled)*	0.71	0.51 - 0.97	**0.037**	0.63	0.3 – 0.91	**0.016**
Observations	125			109		
AIC	155.992			131.814		

(c) Proximal Femur BMD

	RSI Model 5		
Coefficient	*Odds Ratios*	*CI (95%)*	*P*
Age	1.26	1.11 – 1.46	**0.001**
Body fat	1.17	1.04 – 1.34	**0.013**
Diet restriction (Minor)	3.87	1.21 – 1.34	**0.029**
Diet restriction (Considerable)	3.33	1.21 – 14.15	0.072
Female (*Reference*)			
Male	3.84	0.84 – 20.48	0.097
Femur BMD (scaled)*	0.61	0.38 – 0.92	**0.027**
Observations	103		
AIC	122.949		

(d) Female specific

	RSI Model 6		
Coefficient	*Odds Ratios*	*CI (95%)*	*P*
Age	1.43	1.16 – 1.90	**0.004**
Body fat	1.28	1.07 – 1.61	**0.016**
Current menstrual status (Irregular)	13.87	2.62 – 114.04	**0.005**
Rib BMD (scaled)*	0.24	0.04 – 1.15	0.092
Observations	47		
AIC	51.135		

* Values were multiplied by 10 to obtain odds ratios for 0.1 unit difference

Supplementary Table 2 Multiple linear regression models for bone mineral density

(a) Diet restriction on AP spine BMD

	Spine BMD Model		
Coefficient	*Estimates*	*CI (95%)*	*P*
Age	0.01	0.00 - 0.01	**0.032**
Diet restriction (Minor)	-0.06	-0.11- -0.00	**0.040**
Diet restriction (Considerable)	-0.09	-0.15- -0.03	**0.002**
Female (*Reference*)			
Male	0.04	-0.01 – 0.08	0.104
Observations	115		
AIC	-152.870		

(b) Sex, weight category and RSI on proximal femur BMD

	Proximal Femur BMD Model 1			**Proximal Femur BMD Model 2**		
Coefficient	*Estimates*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*
Heavyweight (*Reference*)						
Lightweight	-0.08	-0.13 - -0.04	**<0.001**	-0.08	-0.12 - -0.03	**0.001**
RSI history				-0.05	-0.09 - -0.00	**0.045**
Female (*Reference*)						
Male	0.07	0.03 - 0.11	**0.002**	-0.08	-0.12 - -0.03	**0.001**

Observations	125	115
AIC	-162.981	-152.518

(c) Weight category and age on rib BMD

	Rib BMD Model 1			**Rib BMD Model 1 with calcium**		
Coefficient	*Estimates*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*
Age	-0.00	-0.00 - 0.0)	0.060	-0.00	-0.00 – 0.00	0.743
Female (*Reference*)						
Male	0.08	0.07 - 0.10	**<0.001**	0.08	0.06 – 0.10	**<0.001**
Heavyweight (*Reference*)						
Lightweight	-0.08	-0.10 - -0.07	**<0.001**	-0.08	-0.10 – -0.06	**<0.001**
Calcium (scaled)[#]				-0.00	-0.01 – 0.01	0.946
Observations	111			79		
AIC	-370.104			-267.336		

(d) Diet restriction and training age on rib BMD

	Rib BMD Model 2			**Rib BMD Model 2 with calcium**		
Coefficient	*Estimates*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*
Diet restriction (Minor)	-0.05	-0.08 - -0.01	**0.012**	-0.04	-0.09 – 0.00	0.053
Diet restriction (Minor) – Male	-0.01	-0.05 – 0.04	0.822	0.01	-0.05 – 0.06	0.820
Diet restriction (Considerable)	-0.02	-0.06 – 0.02	0.384	-0.01	-0.06 – 0.04	0.704
Diet restriction (Considerable) – Male	-0.09	-0.15 - -0.04	**0.001**	-0.07	-0.14 – -0.00	**0.041**

Female (*Reference*)						
Male	0.10	0.07 – 0.14	**<0.001**	0.09	0.05 – 0.14	**<0.001**
Training age	-0.00	-0.01 - -0.00	**0.037**	-0.00	-0.01 – 0.00	0.277
Calcium (scaled)[#]				0.01	-0.00 – 0.03	0.105
Observations		105			78	
AIC		-322.849			-229.645	

Supplementary Table 3. RSI and BMD regression models evaluating the impact of calcium, vitamin D and vitamin K.

	RSI Model			**Proximal Femur BMD Model**			**Spine BMD Model**			**Rib BMD Model**			**Rib BMD Calcium Model**		
Coefficient	*Odds Ratios*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*	*Estimates*	*CI (95%)*	*P*
Calcium (scaled)[#]	0.75	0.27 – 1.86	0.557	0.03	-0.03 – 0.08	0.358	0.03	-0.02 – 0.08	0.220	0.05	0.02 – 0.07	**0.001**	0.03	0.01 – 0.05	**0.001**
Vitamin D (scaled)[##]	0.31	0.01 – 12.02	0.539	-0.13	-0.38 – 0.12	0.286	-0.02	-0.24 – 0.21	0.877	0.02	-0.09 – 0.14	0.676			
Vitamin K	0.67	0.25 – 1.59	0.391	-0.03	-0.08 – 0.03	0.325	-0.01	-0.06 – 0.04	0.596	-0.01	-0.03 – 0.02	0.572			
Observations		49			43			43			43			79	
AIC		62.907			-48.128			-56.104			-110.029			-198.829	

[#] Calcium values were divided by 1000 to obtain odds ratios for 1000 unit difference
[##] Vitamin D values were divided by 100 to obtain odds ratios for 100 unit difference

Supplementary Table 4. Case series of participants with history of multiple diagnoses of rib stress injuries

	Number of:		Sex	Bone BMD g/cm^2 (Z score)			Diet restriction	Menstrual status	Calcium, mg	Vitamin D	Comments
	RSF	RSR		Spine	Femur	Rib					
Case 1	2	0	Female	1.159 (-0.6)	1.051 (-0.4)	0.627	Yes	Menarche 15 y Recent: irregular, Historical: Amenorrhoea	2020	113 Optimal	Also recent sacral fracture
Case 2	1	1	Female	1.377 (0.9)	1.184 (0.5)	0.713	Yes	Menarche 14y Recent and historical: irregular	-	91 Optimal	Switching between sweep and scull
Case 3	2	1	Female	1.20 (0.2)	1.11	0.673	No	Menarche 16y Recent and historical: Regular	1413	-	Switching between sweep and scull
Case 4	0	2	Male	1.432 (1.3)	1.143 (0.1)	0.803	No	-	-	47 Low	Returning after back injury, first year in sweep after sculling
Case 5	0	2	Male	1.463 (1.6)	1.175 (0.4)	-	Yes	-	-	60 insufficient	

RSF = Rib stress fracture, RSR = Rib stress reaction

5. Interlinking chapter

In the first study of this thesis associations between BMD, RSI history and related nutrition factors were investigated. Self-reported diet restriction but not vitamin D, K or calcium intake were associated with lower spine and rib bone mineral density. Among rowers with RSI history lightweight males had lower total bone mass, femur and rib BMD and heavyweight females had lower rib BMD. In relation to RSI history, the best models included BMD for rib, spine or femur with age, body fat and sex. In female models menstrual history could be used in place of BMD.

Given the importance of diet restriction in Study 1, the next study we focussed on low energy availability in male athletes and the development and validation of a screening tool for this purpose. Screening tools for female athletes are already available.

6. Screening for low energy availability in male athletes: attempted validation of LEAM-Q

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Screening for Low Energy Availability in Male Athletes: Attempted Validation of LEAM-Q

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Abstract: A questionnaire-based screening tool for male athletes at risk of low energy availability (LEA) could facilitate both research and clinical practice. The present options rely on proxies for LEA such screening tools for disordered eating, exercise dependence, or those validated in female athlete populations. in which the female-specific sections are excluded. To overcome these limitations and support progress in understanding LEA in males, centres in Australia, Norway, Denmark, and Sweden collaborated to develop a screening tool (LEAM-Q) based on clinical investigations of elite and sub-elite male athletes from multiple countries and ethnicities, and a variety of endurance and weight-sensitive sports. A bank of questions was developed from previously validated questionnaires and expert opinion on various clinical markers of LEA in athletic or eating disorder populations, dizziness, thermoregulation, gastrointestinal symptoms, injury, illness, wellbeing, recovery, sleep and sex drive. The validation process covered reliability, content validity, a multivariate analysis of associations between variable responses and clinical markers, and Receiver Operating Characteristics (ROC) curve analysis of variables, with the inclusion threshold being set at 60% sensitivity. Comparison of the scores of the retained questionnaire variables between subjects classified as cases or controls based on clinical markers of LEA revealed an internal consistency and reliability of 0.71. Scores for sleep and thermoregulation were not associated with any clinical marker and were excluded from any further analysis. Of the remaining variables, dizziness, illness, fatigue, and sex drive had sufficient sensitivity to be retained in the questionnaire, but only low sex drive was able to distinguish between LEA cases and controls and was associated with perturbations in key clinical markers and questionnaire responses. In summary, in this large and international cohort, low sex drive was the most effective self-reported symptom in identifying male athletes requiring further clinical assessment for LEA.

Keywords: testosterone; endurance; questionnaire; validation; EHMC

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1. Introduction

Awareness and understanding of the impacts of low energy availability (LEA) in athlete populations has continued to evolve and stimulate research interest. Energy availability (EA) is defined as the amount of dietary energy remaining for all other metabolic processes after the energy cost of exercise has been subtracted [1]. Short term (5 days)

clinical studies in eumenorrheic women have demonstrated that the pulsatility of the luteinising hormone is disrupted when they are exposed to an EA below 126 kJ (30 kcal)/kg fat free mass (FFM)/day [2], although a specific EA threshold below which menstrual disturbances are induced is not supported [3]. While the interplay of LEA with bone health and menstrual function in female athletes is relatively well understood [4], an equivalent understanding in male athletes is still developing. The concept of Relative Energy Deficiency in Sport (RED-S) and the Male Athlete Triad [5] has encouraged researchers and clinicians to explore LEA in both male and female athletes and to look for a broader range of potential consequences [6–8].

Prevalence of LEA in male athletes is relatively undescribed, with early estimates ranging between 25 and 70% in road cyclists, distance and cross-country runners and jockeys [9–13]. Few studies have induced LEA in males in a controlled setting [14–17], instituting LEA at 62 kJ (15 kcal)/kg FFM for a period of 4–6 days and with a limited scope of investigation such as bone or iron metabolism markers. These short periods and thresholds of LEA have not been reflective of perturbations seen with females at a similar level [2], with cross-sectional field studies or severe energy restriction research in males [18]. It is possible males have a higher tolerance to LEA severity and/or duration and gender-specific thresholds are required. It has been noted that male endurance athletes may have chronic lower testosterone levels (40–75% of normal, healthy, age-matched sedentary males), a condition described as the Exercise Hypogonadal Male Condition (EHMC) [19], but whether this is a normal adaptation to training or due to LEA is still under debate [20]. Causation appears to parallel that in female athletic populations with identified contributors, including disordered eating behaviour [21–23], exercise dependence [24] and participation in aesthetic, weight sensitive or endurance sports [25–27].

The causes of LEA are multi-factorial and include a misunderstanding of the energy needs for sport, limitations of food availability, dietary restraint, and overzealous weight loss programs (including excessive amounts of exercise), and disordered eating or eating disorders [6]. Regardless of origin, LEA can act as a serious impediment to good health and sport performance [28–30]. Indeed, there is increasing evidence that exposure to LEA in male athletes is associated with effects on the hypothalamic-pituitary-gonadal (HPG) axis [9,31–41], changes to immune function [39,42] impairments of bone health [43–45] and reproductive function [46], and negative outcomes for performance [10,42] and body composition [47].

These limitations aside, the identification and appropriate management of LEA is a core competency for practitioners who work with athletic populations. A quantitative assessment of EA from measurements of energy intake, exercise energy expenditure and fat free mass is time consuming and impractical as a broad-scale screening tool for use by clinicians. Of greater importance, such assessments are fraught with potential errors or misrepresentation [48], making research in this area more challenging. Surrogate markers of EA may provide alternative ways to assess athletes for risk of the health and performance consequences of LEA [49]. The measurement of resting metabolic rate (RMR) is an accepted method [50–52] but requires technical skill and equipment and is also impractical on a large scale. Low bone mineral density (BMD) is often seen in those presenting with LEA (reviewed in [53]) but may not differentiate between current LEA and previous exposure that may have been resolved. Blood markers, including changes to hormones such as testosterone, insulin-like growth factor-1 (IGF-1), triiodothyronine (T_3), insulin, blood lipids, leptin, and cortisol have been associated with LEA in males [14,18,32,54–58] but are beyond the budget for most sport organisations, teams or clubs to use routinely. Given this, there is interest in the development of a screening tool that could help triage those male athletes requiring specific follow-up to investigate LEA.

Screening questionnaires provide a framework to assess groups to identify those at risk and requiring further follow up. The Low Energy Availability among Females Questionnaire (LEAF-Q) is a screening questionnaire for LEA which was developed in a female endurance athlete population [59]. It provides an opportunity to triage a larger group of athletes to identify those requiring further follow up or as a simple way to track

changes in individuals or groups over time. Since publication, this questionnaire has been used clinically and in research settings to assess prevalence of LEA risk and consequences in different populations [60–65] and has encouraged awareness and further research in this area.

A variety of approaches have been used in male athletic populations as a proxy for clinical identification of LEA, such as the exercise dependence scale (ExDS) [24] or eating disorder questionnaires, such as the eating disorder examination questionnaire (EDE-Q) [66]. The male and female athlete triad coalition have recommended a series of questions to screen for the male athlete triad along with a cumulative risk assessment (CRA) tool adapted from females, excluding the menstrual cycle questions [5]. Whilst these questions have good scientific logic, they are intended to identify bone health and eating disorder risk rather than LEA, per se, and have not been validated for this purpose. This modified CRA has been used successfully to assess the risk of bone stress injury [25]. Others have used the LEAF-Q with the menstrual function section removed and scores adjusted to allow for the lower number of questions [67] or replacing the menstrual function questions with those around sex drive and morning erections [66]. In a large-scale study by Hackney et al., a combination of validated questionnaires regarding physical characteristics, training and sex drive demonstrated that higher training loads are predictive of lower sex drive; however, EA was not considered [68]. Similarly, the Androgen Deficiency in Aging Males questionnaire (ADAM-Q) [69] has been used to identify male athletes with changes to their reproductive function [70], but it is unclear whether the symptoms identified are due to LEA or other causes such as chronic endurance training [68]. The Sport Specific Energy Availability Questionnaire and Interview (SEAQ-I) [10] is a questionnaire and clinical interview developed for male cyclists but relies on practitioner expertise for use and has been assessed for content validity only. It assumes LEA based on reported energy restriction and weight change. The validation process for the RED-S Specific Screening Tool (RST) [71] was inadequate, correlating scores against the pre-participation gynaecological examination [72], which itself has not been validated and was developed for adolescent females and without sufficient attention to sex differences in presentation of LEA symptoms. The Dance Specific Energy Availability Questionnaire (DEAQ) [26] utilizes questions from previously validated questionnaires including LEAF-Q and ADAM-Q [69], as well as questions used in the RED-S Clinical Assessment Tool (RED-S CAT) [73] and SEAQ-I [10]; however, these have not been validated to identify LEA in male athletic populations, either separately or in the current format.

In summary, despite the obvious interest and need for both clinicians and researchers [7,66], a validated questionnaire that could be used as a screening tool for LEA in male athletes does not currently exist. Accordingly, this study aimed to use clinical markers associated with LEA in males to develop and validate a screening tool, the Low Energy Availability among Males Questionnaire (LEAM-Q) for adult sub-elite to elite male athletes.

2. Materials and Methods

A total of 405 male athletes were recruited in a multi-centre study, undertaken as a collaboration between the Australian Institute of Sport, the Norwegian Olympic and Paralympic Committee and Confederation of Sports, the University of Copenhagen and the University of Agder. Inclusion criteria were elite and sub-elite male athletes, 18–50 years old with an absence of thyroid or metabolic disease. All subjects received information regarding the background of the study, test procedures and signed an informed consent document. Ethics approvals for each testing site were granted by the Australian Institute of Sport Ethics Committee, the Capital Region of Denmark, the University of Agder's Faculty Ethics Committee, the Norwegian Regional Committees for Medical and Health Research Ethics and the Norwegian Centre for Research Data (NSD). The questionnaire was created using content from the LEAF-Q [59], ADAM-Q [74], REST-Q [75], literature review and expert consultation for content validity. Each question was scored on a Likert-type ordinal or nominal scales, with a higher score indicating a greater likelihood of LEA.

The validation was assessed in a two-step process, first for internal consistency and reliability in a young adult male athlete population (n = 53) and secondly, in a separate participant group, described below, to verify the self-reported symptoms from the questionnaire against measured clinical markers associated with LEA (n = 352). The questionnaire initially included 33 items covering dizziness, gastrointestinal function, injury and illness and wellbeing and recovery. The questionnaire was revised part way through collection and increased to 42 items, with additional questions on dizziness, wellbeing and recovery, sleep and sex drive. Sex drive questions were initially not included, in view of expert advice that the questionnaire should be comfortable to administer and discuss across a range of male athlete populations from different cultural backgrounds. After reviewing the initial results, however, questions on sex drive were added to improve sensitivity of the questionnaire. Both versions of the questionnaire included questions to provide demographic and athletic status information. Supplementary File S1 shows the initial version of the questionnaire prior to analysis, with questions added during the revision highlighted in red (version 1). Supplementary File S2 shows the final questionnaire (version 2) and associated scoring key, with sex drive being the sole section retained.

2.1. Internal Consistency and Reliability

To assess the performance of individual items and estimate reliability, a test–retest was performed. Forty-two male athletes were recruited from Australia, Norway and Sweden and received the LEAM-Q (Version 2) in either English, Norwegian or Swedish, as appropriate. The participants were asked to complete the questionnaire twice, 14 days apart. After the re-test, researchers asked the participants to identify any concerns they had with the items, including ease of understanding, relevance, and the appropriateness of the possible answers. Questionnaires were identified by subject number only and were collected either on paper or secure electronic format; Microsoft Forms or SurveyXact, (8200 Aarhus, Denmark).

2.2. Clinical Verification of Self-Reported Symptoms

A cohort of 352 male athletes was recruited for the main activity of the study, representing sports designated as weight sensitive (lightweight rowing, race walking, triathlon, road cycling, marathon, gymnastics, and ballet) or non-weight sensitive (openweight rowing, gymnastics, athletics, other). The LEAM-Q was completed online or on paper by all participants, with 42 ultimately removed due to missing key data. This resulted in 310 participants being involved in the final analysis of the LEAM-Q outcomes (Version 1: 183; Version 2: 127) against clinical assessment.

For the assessment of clinical markers, participants met at their respective test centre between 5 and 9 a.m. in a rested, fasted state (no food or fluid intake or prior physical activity on the morning of the assessment). Body mass was measured to the nearest 100 g and height to the nearest millimetre using calibrated instruments at the different centres. Body composition and BMD were assessed using Dual-energy X-ray absorptiometry (DXA) in the total body and site-specific modes on a narrow fan-beam DXA scanner (GE-Lunar Prodigy or iDXA, using GE enCORE analysis software version 15.0 or 16.2, Madison, WI, USA). Protocols included appropriate machine calibration and standardised positioning and were in keeping with best practice guidelines as previously described [76,77]. BMD was assessed for proximal femur and anterior posterior lumbar spine (L1–L4). For the Scandinavian cohorts the combined NHANES/Lunar reference database was used and for the Australian cohort the combined Lunar/Geelong as deemed most appropriate for the respective populations and low BMD was defined in Table 1.

RMR was measured either by metabolic cart (Oxycon Pro or Vyntus CPX, Jaeger GmbH, Hoechberg, Germany) or the first principles method [78] involving Douglas bags [78], depending on the testing location. All measures replicated participant preparation and presentation and were collected in a warm, quiet, and dimly lit room. For the metabolic cart method, a ventilated canopy hood system was used to assess RMR, with systems being

calibrated before each test according to standards, and alcohol calibration weekly. Subjects rested for 15 min prior to collection. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were assessed over a 30 min period and converted to kJ/min based on the Weir equation [79]. The last 20 min of measurement were used to assess RMR using the protocol defined by Compher et al. [80]. The first principles method replicated the processes as previously described [81]. To calculate the RMR_{ratio}, the Cunningham (1980) equation [82] was used to calculate the predicted RMR of each subject: 500 + (22 × LBM [kg]). Resting Energy Expenditure (REE) was also calculated relative to fat free mass (FFM) as determined by DXA (kJ/kg FFM). As systematic differences were noted between the first principles and metabolic cart measurements and, as no threshold for RMR_{ratio} has been identified for male athletes, the lowest quartile of each method was used to indicate a "low RMR" finding (Table 1).

Blood pressure (BP) was obtained in a resting supine position using an electronic sphygmomanometer (Microlife BP A100, Widnau, Switzerland or HEM7320, Omron Healthcare, JA Davey Pty Ltd., Melbourne, VIC, Australia). The monitor was secured around the participant's left upper arm, and automatically provided a reading of systolic and diastolic blood pressure, and resting heart rate.

Blood samples were collected within 30 min of completion of the RMR measurement, obtained via venepuncture from an antecubital forearm vein by a qualified phlebotomist. This ensured samples were fasted, rested and collected at a similar time of day for all participants [83]. For the Scandinavian cohorts, blood was clotted at room temperature for 30 min before being centrifuged at 1300× *g* for 10 min. Serum was transferred into tubes and stored at −80 °C until analyses. The serum from Kristiansand was analysed at St. Olavs Hospital (Trondheim, Norway) and serum from Oslo was analysed at Fürst medical laboratory (Oslo, Norway), for its content of glucose (CV 1.6%), insulin, cortisol (CV 3–5.4%), total testosterone (CV 6–9.2%), free triiodothyonine (T_3) (CV 2.3–4.7%), and IGF-1 (CV4.8–7.5%). For the Australian cohort a single venous blood sample (2 × 8.5 mL serum separator tube) was used for the assessment of fasting IGF-1, cortisol, lipids, insulin, testosterone and T_3 for analysis by chemiluminescent immunoassay through a commercial laboratory (Laverty Pathology, Bruce, ACT, Australia). IGF-1 was assayed using the DiaSorin Liason® XL (DiaSorin Diagnostics, Sallugia, Italy, CV 2.5–6.4%), whilst cortisol (CV 2.9–5.2%), testosterone (CV 4.5–8.2%) and T_3 (CV 2.6–5.3%) were assayed using the Siemens ADVIA Centaur XP (Siemens Healthcare Diagnostics Ltd., New York, NY, USA) as per the manufacturer's recommendations. Fasting blood glucose levels were assessed via fingertip capillary sample using a portable meter and test strip (Accu-Chek® Performa, Roche Diagnostics, Castle Hill, NSW, Australia, CV < 5%). Free testosterone was calculated from total testosterone, sex hormone binding globulin and albumin, or where unavailable 43 g/L, according to the method by Vermeulen et al. [84]. As the blood analyses were conducted at different laboratories, a "low" finding was determined using the lowest quartile of the reference range for the laboratory at which the measure was taken (Table 1).

2.3. Statistics

To assess the performance of the items and estimate reliability, the intraclass correlation coefficient (ICC) was used to calculate the difference between the test and the retest score using a two-way mixed random effects model.

The association between clinical outcomes and LEAM-Q variables were assessed including all subjects (n = 310), using multivariate linear or logistic regression models for all combinations of clinical outcomes (as responses) and screening variables from LEAM-Q (as predictors), including adjustment for age, BMI, elite athlete (yes/no) and centre (if there were data from multiple centres) (Table 2). In addition to the standard questionnaire scoring, a separate score was conducted for symptoms included in the EHMC [85]. This was assessed as a score for the sex drive questions "In general I would rate my sex drive as", "Morning erections over the last month" and "How many morning erections compared to normal" in combination with the items "I feel tired from work or school", "I feel lethargic",

"I feel strong and making good progress with my strength training", "I feel very energetic in general", I feel invigorated for training sessions and ready to perform well", "I feel happy and on top of my life outside of sport". Low sex drive was also categorised by using sex drive scores equal or greater than 2 on "Sex drive in general" or equal or greater than 2 on "The number of morning erections" and equal or greater than 1 for "Morning erections compared to normal" to represent reproductive dysfunction. Weight flux was defined by the difference between "highest" and "lowest body weight at current height" responses from the questionnaire.

ROC curves were used for evaluating, optimizing, and visualizing the performance of classifications of a continuous biomarker into two groups for predicting the clinical outcome of interest, LEA [86]. For LEAM-Q variables with significant association to one or more clinical outcomes ($p < 0.05$), optimal sensitivity was estimated using ROC curve analysis with Youden's index [87]. At least 60% sensitivity was required to identify potentially useful screening variables, which were retained (Table 3). For clinical variables classified as "high" or "low", this represented the test locations highest or lowest 25% percent of results, respectively (Table 1). Data were analysed using R (R Core Team 2020. R Foundation for Statistical Computing, Vienna, Austria) with the following extension packages, Hmisc [88] and pROC [89].

Given the recognised limitations of EA assessments in the field [5], LEA was operationally defined as having two or more primary indicators or three or more indicators overall. Primary indicators were derived from the male athlete triad [5] and secondary indicators from energy restriction and LEA literature [5,7,59,90–92]. After excluding 45 subjects missing at least three clinical markers, including at least one primary clinical marker, the remaining subjects ($n = 265$) were categorised as LEA-cases or controls by using the criteria outlined in Table 1. A two-sample *t*-test was used to analyse differences in the retained LEAM-Q variables between cases and controls (Table 4).

Table 1. Definition of Clinical Indicators of LEA.

Primary Indicators	Secondary Indicators
1. **Low T_3:** lowest quartile * (<3.5 pmmol/L) [5]. 2. **Low total or free testosterone:** Lowest quartile (<16 nmol/L, <333 pmol/L, respectively) [5]. 3. **Low BMD:** Z-score <−1 for either AP spine or proximal femur [5,7]. 4. **Low body weight:** Body Mass Index (BMI) <18.5 kg/m^2 [5,91].	1. **Low RMR_{ratio}** [5] lowest quartile for the testing method (<1.11 for first principles and <0.88 for metabolic cart measures). 2. **Hypotension:** <90 mmHg systolic and/or diastolic <60 mmHg [59]. 3. **Low body fat:** <5% as measured by DXA [92]. 4. **Low IGF-1:** lowest quartile of the age dependent reference range at the testing site [90]. 5. **High LDL cholesterol** (>3mmol/L) [59]. 6. **High cortisol** (>550 nmol/L) or cortisol (nmol/l) insulin ratio (pmol/l) (>26.6).

Subjects were categorized as LEA if they had two or more primary indicators or three or more indicators overall. * "Lowest quartile" refers to the lowest quartile of the reference range at the specific testing site where the measure was taken.

Table 2. Multivariate analysis of questionnaire items and associated clinical markers.

Questionnaire Item	Clinical Variable	N	Estimated Slope	SE	*p*-Value
Section 1: Dizziness					
1. Dizziness score	Glucose	264	−0.075	0.032	0.018
	Low insulin	117	0.600	0.227	0.008
	Proximal Femur BMD Z-score	302	−0.196	0.063	0.002
	High cortisol:insulin ratio	95	0.513	0.219	0.019

Table 2. *Cont.*

Questionnaire Item	Clinical Variable	N	Estimated Slope	SE	*p*-Value
Section 2: Gastrointestinal Score					
2. Gastrointestinal score	AP Spine BMD Z-score	304	−0.136	0.0394	0.004
	Proximal Femur Z-score	302	−0.078	0.039	0.046
Section 3: Thermoregulation- no findings					
Section 4: Injury and illness					
4A How many acute injuries?	Low T_3	177	0.683	0.279	0.014
	T_3	177	−0.140	0.059	0.019
4B How many overload injuries?	Low T_3	177	0.537	0.230	0.020
	T_3	177	−0.130	0.054	0.018
	High cortisol	207	0.391	0.190	0.039
	High cortisol:insulin ratio	95	0.506	0.238	0.034
4D How many breaks in training have you had for acute injury?	High cortisol	209	0.389	0.168	0.021
	Cortisol	209	22.725	9.856	0.022
4F Number of days unable to train due to illness	Low T_3	176	0.762	0.267	0.004
	T_3	176	−0.191	0.054	0.001
4 Injury and illness score	Low T_3	177	0.173	0.065	0.008
	T_3	177	−0.038	0.014	0.008
	High cortisol	217	0.093	0.045	0.040
Section 5: Wellbeing and recovery					
5A Fatigue sub score	Total cholesterol	241	0.048	0.022	0.028
5D Poor recovery sub score	Total cholesterol	241	0.078	0.031	0.013
5E Low energy levels	Low insulin	117	0.2133	0.098	0.030
5 Poor wellbeing score	Total cholesterol	241	0.016	0.006	0.013
Section 6: Sex Drive					
6A How would you rate your sex drive in general?	High cortisol:insulin ratio	95	0.767	0.373	0.039
	Weight flux	115	1.908	0.579	0.001
	Training amount	114	7.995	4.010	0.049
	Low insulin	95	1.177	0.416	0.005
	Cortisol:insulin ratio	95	4.959	1.897	0.011
	Total Testosterone	115	−1.882	0.826	0.025
	Proximal femur BMD Z-score	112	−0.326	0.130	0.014
	T_3	114	−0.195	0.090	0.033
6B How would you rate it over the last month compared to normal?	T_3	114	−0.221	0.106	0.039
	Glucose	107	−0.172	0.077	0.027
	Low insulin	95	0.817	0.398	0.040
6C How often would you wake with a morning erection?	AP Spine BMD Z-score	115	−0.177	0.074	0.019
	Training amount	114	4.734	2.265	0.039
	Low free testosterone:cortisol ratio	114	0.4346	0.1946	0.026
	Proximal femur BMD Z-score	112	−0.228	0.073	0.002
	Low BMD	115	0.520	0.211	0.014
6D Over the last month how does the number of morning erections compare to normal for you?	Low RMR_{ratio}	115	0.743	0.343	0.030
Low sex drive score	High cortisol:insulin ratio	95	0.206	0.103	0.045
	Weight flux	115	0.4819	0.180	0.009
	Low insulin	95	0.209	0.105	0.045
	Proximal femur BMD Z-score	112	−0.121	0.039	0.003
	Testosterone	115	−0.5874	0.2527	0.022
	T_3	114	−0.074	0.028	0.009

Table 2. *Cont.*

Questionnaire Item	Clinical Variable	N	Estimated Slope	SE	*p*-Value
Exercise Hypogonadal Male Condition	Weight flux	118	2.049	0.887	0.023
	Proximal femur BMD Z-score	115	−0.397	0.193	0.042

Significance set at $p < 0.05$, $n = 310$. "High" represents the top and "low" represents the bottom quartile of the test locations' clinical variables, respectively.

Table 3. ROC analysis including all subjects ($n = 310$) showing questionnaire items associated with clinical variables according to the multivariate analysis (Table 1) with a sensitivity of >60%.

Questionnaire Item	Associated Clinical Variable	Score Threshold	Sensitivity (%)	Specificity (%)
1 Dizziness score	High cortisol:insulin ratio	0.5	70	52
	Glucose	0.5	62	49
	Low insulin	0.5	70	54
4F Illness score	Low T_3	0.5	64	46
	T_3	0.5	67	47
5 Poor wellbeing score	Total cholesterol	19.5	61	56
5A Fatigue	Total cholesterol	2.5	82	31
6 Low sex drive score	T_3	1.5	64	86
	Low insulin	0.5	96	28
	Weight flux	0.5	81	24
6A Sex drive in general	Total testosterone	0.5	87	26
	Weight flux	1.5	69	56
6B Sex drive over the last month	T_3	2.0	71	98
6C Morning erections	Low free testosterone:cortisol ratio	0.5	63	57

Table 4. Subject characteristics LEA cases vs. controls.

Variable	All ($n = 310$)	Controls ($n = 180$)	LEA-Cases ($n = 85$)	*p*-Value
Age (years)	27.9 ± 6.9	27.0 ± 6.7	31.2 ± 7.6	<0.0001
Age at specialization (years)	18.1 ± 7.7 ($n = 303$)	17.9 ± 7.1 ($n = 177$)	21.3 ± 8.6 ($n = 77$)	0.0010
Height (cm)	181.6 ± 7.7	182.1 ± 8.4	180.5 ± 6.5	0.1232
Body mass (kg)	73.4 ± 10.1	74.9 ± 11.0	72.1 ± 9.3	0.0449
BMI (kg/m^2)	22.2 ± 2.0	22.5 ± 2.0	22.1 ± 2.1	0.1256
Weight flux (max min weight)	9.1 ± 9.5	8.9 ± 5.7	10.1 ± 6.5	0.1390
VO_{2max} (mL/kg/min)	68.1±7.2	67.9 ± 7.1 ($n = 129$)	67.9 ± 7.4 ($n = 71$)	0.9369
DXA body fat %	11.9 ± 3.8	12.5 ± 3.5	12.3 ± 3.7	0.6941
DXA FFM (kg)	64.9 ± 8.7	65.7 ± 9.7	63.7 ± 7.6	0.1050
AP Spine BMD Z-score	−0.01 ± 1.00 ($n = 259$)	0.05 ± 1.03 ($n = 174$)	−0.28 ± 1.01	0.0147
Proximal Femur BMD Z-score	0.35 ± 1.0 ($n = 257$)	0.31 ± 0.96 ($n = 173$)	0.04 ± 0.92 ($n = 84$)	0.0325
BP systolic (mmHg)	118.6 ± 10.4 ($n = 247$)	119.9 ± 10.7 ($n = 149$)	116.9 ± 9.7 ($n = 76$)	0.0373
BP diastolic (mmHg)	67.6 ± 7.6 ($n = 247$)	68.1 ± 6.5 ($n = 149$)	67.3 ± 6.5 ($n = 149$)	0.4088
RMR (kJ/kg FFM)	125.7 ± 16.3 ($n = 286$)	130.8 ± 15.1	120.1 ± 14.9 ($n = 82$)	<0.0001
RMR_{ratio}	1.01 ± 0.13 ($n = 288$)	1.05 ± 0.12	0.95 ± 0.12 ($n = 83$)	<0.0001
Total testosterone (nmol/L)	19.8 ± 5.8 ($n = 256$)	21.2 ± 5.5 ($n = 168$)	17.3 ± 5.5 ($n = 83$)	<0.0001
Free testosterone (pmol/L)	425.3 ± 139.1 ($n = 207$)	456.4 ± 136.2	383.7 ± 136.8	0.0008

Table 4. *Cont.*

Variable	All (n = 310)	Controls (n = 180)	LEA-Cases (n = 85)	p-Value
Free testosterone:cortisol ratio	1.01 ± 0.47 (n = 199)	1.10 ± 0.46 (n = 127)	0.87 ± 0.43 (n = 727)	0.0006
Total testosterone:cortisol ratio	0.05 ± 0.02 (n = 217)	0.05 ± 0.02 (n = 139)	0.04 ± 0.02 (n = 78)	0.0002
IGF-1 (nmol/L)	28.7 ± 8.5 (n = 218)	31.5 ± 8.3 (n = 123)	24.8 ± 7.5 (n = 75)	<0.0001
T_3 (pmol/L)	5.3 ± 0.8 (n = 177)	5.7 ± 0.5 (n = 104)	4.9 ± 0.7 (n = 53)	<0.0001
Cortisol (nmol/L)	461.5 ± 127.5 (n = 217)	449.0 ±121.9 (n = 139)	483.9 ± 134.7 (n = 78)	0.0523
Insulin (pmol/L)	24.2 ±10.3 (n = 117)	26.4 ± 10.9 (n = 61)	20.8 ± 7.4 (n = 36)	0.0079
Cortisol:insulin ratio	22.1 ± 14.5 (n = 95)	19.3 ± 10.1 (n = 61)	27.1 ± 14.7 (n = 34)	0.0031
Blood glucose (mmol/L)	5.0 ± 0.4 (n = 264)	5.1 ± 0.5 (n = 168)	4.9 ± 0.5 (n = 74)	0.0893
Total cholesterol (mmol/L)	4.6 ± 0.9 (n = 241)	4.5 ± 0.8 (n = 159)	4.8 ± 0.9 (n = 80)	0.0292
LDL (mmol/L)	2.7 ± 0.8 (n = 239)	2.7 ± 0.7 (n = 159)	2.9 ± 0.8 (n = 78)	0.0680
HDL (mmol/L)	1.5 ± 0.3 (n = 240)	1.4 ± 0.3 (n = 159)	1.5 ± 0.4 (n = 79)	0.0303
Triglycerides (mmol/L)	0.9 ± 0.3 (n = 241)	0.94 ± 0.34 (n = 159)	0.89 ± 0.37 (n = 80)	0.2783

Data are expressed as mean ± standard deviation, significance set at $p < 0.05$.

3. Results

3.1. Questionnaire Validation Process

Two items were removed from further analysis following the test–re-test process due to low ICC ("How would you describe your normal stool" and "I feel down and less happy that I used to feel or would like to feel"). Following this revision, fourteen-day test–re-test reliability ICC was 0.71. [59].

3.2. Subject Characteristics for Main Analysis

Of the 310 participants included in the analyses, 64% were elite athletes, 31% sub elite and 5% club level athletes from ten different countries. Half of these participants reported being full time athletes or professional, with 36% reporting placing within the top 10 at their respective international competition. Based on the definition summarised in Table 1, 2% of participants were classified as underweight, while none had low body fat levels, 24% had low BMD, 27% had low RMR, and low blood concentrations were found for testosterone (23%), T_3 (17%) and insulin (26%). High blood cortisol concentrations were found in 28% of participants, while 30% had high LDL cholesterol. Meanwhile 2% of participants had hypoglycaemia and 11% had hypotension. Those who were underweight (n = 5) showed greater weight flux, lower T_3, total testosterone, systolic BP, higher dizziness scores and less morning erections than the rest of the cohort (all $p < 0.05$). Those with hypotension showed no differences with any clinical variable or questionnaire score. Mean maximum oxygen uptake (VO_{2max}) was 68.1 ± 7.2 mL/kg/min. Athletes from a weight sensitive sport had a lower height (179.8 ± 6.8 vs. 188.1 ± 7.3 cm, $p < 0.001$), body mass (71.1 ± 7.8 vs. 82.4 ± 12.7 kg, $p < 0.001$), BMI (21.9 ± 1.8 vs. 23.1 ± 2.4 kg/m^{2,} $p < 0.001$), FFM (62.4 ± 6.8 vs. 73.6 ± 9.6 kg, $p < 0.001$), RMR_{ratio} (0.98 ± 0.13 vs. 1.13 ± 0.13, $p < 0.001$), spine BMD Z score (−0.12 ± 0.97 vs. 0.41 ± 1.0. $p < 0.001$) systolic BP (117 ± 9.7 vs. 127 ± 10.3 mmHg, $p < 0.001$), and higher percent body fat (12.4 ± 3.8 vs. 10.1 ± 3.3%, $p < 0.001$) and T_3 (5.4 ± 0.7 vs. 5.0 ± 0.97 pmol/L, $p < 0.01$) than those from non-weight sensitive sports. No trend was seen for a decline in free or total testosterone with increasing age.

3.3. Case Control Comparison

Forty-five subjects were removed from the classification into LEA-case or control based on incomplete clinical indicators, leaving 265 remaining subjects for this portion of the analysis (Table 4). Of these, 85 (32%) were classified as having LEA. LEA-cases were older,

had a higher age of sport specialisation, lower spine and total femur BMD Z scores, systolic BP, RMR, total and free testosterone, free testosterone:cortisol ratio, IGF-1, T_3, insulin and higher cortisol:insulin ratio, and total and HDL cholesterol compared to controls.

Sub section and total LEAM-Q scores were not different between LEA cases and control cohorts, with the exception of the sex drive score (Table 5). Of the 118 athletes answering the sex drive questions, 23.7% (n = 28) were categorised as having a low sex drive with lower total testosterone (18.0 ± 6.0 vs. 20.9 ± 5.6 nmol/L, p = 0.025), T_3 (5.3 ± 0.7 vs. 5.6 ± 0.7 pmol/L, p = 0.047), and insulin levels (21.1 ± 10.3 vs. 25.8 ± 9.8 pmol/L, p = 0.045), lower femur BMD Z-score (−0.02 ± 0.97 vs. 0.39 ± 0.88, p = 0.041), and diastolic BP (64.7 ± 4.8 vs. 67.9 ± 7.6 mmHg, p = 0.044), while having a higher cortisol:insulin ratio (26.9 ± 17.4 vs. 20.6 ± 10.1, p = 0.035), and weight flux (10.2 ± 5.8 vs. 8.1 ± 4.1 kg, p = 0.037) compared with athletes with a normal sex drive. There was a non-significant trend towards lower free testosterone (0.8 ± 0.5 vs. 1.0 ± 0.4, p = 0.089), IGF-1 (29.4 ± 6.2 vs. 32.2 ± 7.1 nmol/L, p = 0.073), and testosterone:cortisol ratio (0.04 ± 0.02 vs. 0.05 ± 0.02, p = 0.074).

Table 5. Variable scores in LEA cases and controls.

Questionnaire Item	Control (n = 180)	LEA Case (n = 85)	p-Value
1 Dizziness score *	0.8 ± 0.8	0.8 ± 1.0	0.7738
4F Illness score *	0.92 ± 0.98	0.76 ± 0.91	0.1997
5A Fatigue score *	4.48 ± 2.74	3.84 ± 2.76	0.0764
5 Wellbeing score *	18.71 ± 10.89	20.37 ± 10.32	0.2308
6 Low sex drive score *	1.96 ± 1.93 (n = 77)	3.00 ± 2.51 (n = 38)	0.0160
6A Sex drive in general *	0.86 ± 0.58	1.11 ± 0.80	0.0599
6B Sex drive over the last month *	0.17 ± 0.47	0.32 ± 0.34	0.1979
6C Morning erections *	0.75 ± 1.07	1.26 ± 1.33	0.0284
6D Over the last month how does the number of morning erections compare to normal for you? *	0.18 ± 0.62	0.32 ± 0.74	0.3102

* A higher score indicates a clinically less favourable presentation of symptoms.

3.4. Utility of Clinical Variables

Table 6 describes differences in clinical and questionnaire variables between subjects classified as having low testosterone, RMR, T_3, BMD and a high cortisol:insulin ratio compared to those having normal levels. Those classified as having low testosterone, RMR or T_3 had a lower body mass, BMI, and systolic BP. FFM, total testosterone to cortisol ratio, free T_3, systolic BP, and higher cortisol to insulin ratio. Those with low RMR_{ratio} were older, had lower height, body mass, BMI, FFM, systolic BP, total testosterone, IGF-1 and free T_3 levels, and reported less frequent than normal morning erections. Those with low free T_3 (n = 24) had lower RMR (kJ/kg FFM) and RMR_{ratio}, free and total testosterone, free and total testosterone to cortisol ratio, IGF-1 and higher cortisol levels compared to their counterparts with normal free T_3. Those with lower BMD showed no differences in key clinical markers of LEA compared to those with normal BMD. High LDL had no association with clinical markers thought to be indicative of LEA.

Table 6. Utility of clinical variables [1].

Low Testosterone (*n* = 66)	Low RMR_{ratio} (*n* = 71)	Low T_3 (*n* = 46)	Low IGF-1	High Cortisol (*n* = 60)	High Cortisol: Insulin Ratio (*n* = 27)	Low BMD (*n* = 63)	Underweight (*n* = 5)	High LDL (*n* = 73)
Physique and Clinical markers								
Lower Height *, BM **, BMI **, FFM * F and T testosterone:cortisol ratio *** T_3 *** Systolic BP * **Higher** HDL *	**Lower** Height ***, BM ***, BMI *, FFM *** T testosterone * T_3 *** IGF-1 ** Systolic BP ** **Higher** Age *** BMD femur Z-score *	**Lower** BM **, BMI **, % body fat * F and T testosterone *** F testosterone:cortisol ratio *** Systolic and diastolic BP ** Diastolic BP * **Higher** HDL *	**Lower** RMR *** **Higher** Age *** Weight flux *** % body fat ** HDL *	**Lower** % body fat * F testosterone *** F and T testosterone:cortisol ratio *** T_3 *** Cortisol:insulin ratio *** Total cholesterol *	**Lower** % body fat * F testosterone ** F and T testosterone:cortisol ratio ** T_3 ** Glucose * **Higher** Weight flux *	**Lower** None **Higher** TG **	**Lower** T testosterone * Systolic BP ** **Higher** Weight flux *	**Lower** Cortisol ** **Higher** Age ** T testosterone:cortisol ratio * Total cholesterol *** TG **
Questionnaire scores [2]								
Higher poor recovery score * Lower injury and illness score *	Fewer morning erections compared to normal **	Lower general sex drive score *, lower GI score	Lower poor fitness score *** Lower fatigue score *** Lower Wellbeing score ***	Higher Injury and illness score *	Increased dizziness * Lower general sex drive *	None	Higher poor fitness score * Fewer morning erections compared to normal *** Higher dizziness score *	**None**

[1] Definitions of "low" clinical markers defined in Table 1; [2] lower questionnaire score indicates a more normal response; higher scores suggest perturbations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. RMR: resting metabolic rate; FFM: fat free mass; BP: blood pressure; BMD: bone mineral density; BM: body mass; BMI: body mass index; TG: triglyceride; F and T testosterone: free and total testosterone; HDL: high density lipoprotein; T_3: free triiodothyronine, IGF-1: insulin like growth factor one.

4. Discussion

Despite widespread interest, this is the first large scale attempt to validate a specific LEA screening tool for male athletes. Associations were seen between the LEAM-Q questions and clinical markers of LEA with adequate sensitivity in areas of dizziness, illness, wellbeing and fatigue and sex drive. Apart from sex drive, the developed questionnaire was, however, unable to distinguish between LEA cases or controls, as categorised by the researchers, for total score or any sub-score. This is an important finding given the number of questionnaires currently used to identify LEA in male athletes that are either validated only in females or not validated at all. Those classified as having low sex drive by the LEAM-Q questionnaire demonstrated multiple perturbations in clinical markers of LEA. A secondary finding was that perturbations in clinical markers of LEA tended to "cluster" but did not present uniformly across cases. The presentation of male athletes with LEA was different to characteristics shown in the literature on female athletes with LEA, both in the pattern of the questionnaire responses and the clinical markers.

Responses to the LEAM-Q questionnaire found several associations between subscores and perturbations in individual clinical markers. For example, sex drive was associated with total testosterone, T_3, insulin and free testosterone:cortisol ratio, while weight flux was associated with cortisol:insulin ratio, dizziness was associated with glucose and insulin and insulin:cortisol ratio, illness was associated with T_3, and wellbeing and fatigue were associated with high total cholesterol.

The LEAF-Q for LEA in females found an association between gastrointestinal symptoms and characterized LEA [59]. In contrast, the male participants categorised as cases in

the present study did not have higher gastrointestinal scores than the controls, although participants with low T_3 and low spine Z-scores did have higher scores. The physiological basis for an association between gastrointestinal symptoms and BMD is unclear. Gastrointestinal symptoms have been previously associated with self-reported exercise dependence and disordered eating scores in male athletes [66], and in male eating disorder populations [93]. Although there is a possibility of a sex-difference, gastrointestinal symptoms may also be more linked to the athlete's sport type. Indeed, a mixed sport cohort of female athletes did not show links between gastrointestinal symptoms and LEA [94] previously reported in the LEAF-Q validation in endurance and weight sensitive sports [59].

Our study failed to show an association between clinical variables and questions around sleep or thermoregulation, and further research on these themes seems less likely to be productive. Although injury scores were associated with several of our biomarkers of LEA (Table 2), the sensitivity of these scores was low. Indeed, unlike the LEAF-Q validation and other studies in female athletes [59,95], our study failed to find an association between injury scores and BMD [66]. Typically, studies in both male and female endurance athletes have found correlations between bone stress injury rates and BMD [96], with one investigation of male athletes reporting that a cumulative risk assessment score incorporating both LEA and BMD [25] was predictive for bone stress injuries [25]. However, we note the lack of association between LEA and injury in a large scale, mixed sport female population [49] and suggest that in studies involving a diversity of sports, such as the present investigation, injury causation is likely to be multifactorial and less tightly related to LEA. It is possible that more targeted questions around injury within a uniform athlete group may improve the sensitivity of this factor in predicting LEA, but this would also reduce the applicability of the questionnaire across sports as is noted for the LEAF-Q [94]. Failure to find relationships between BMD, LEAM-Q questions and other markers of LEA in the current cohort may be due to the disassociation between acute measurements and the chronic nature of bone health [97,98].

Questions around dizziness were included in the LEAM-Q battery although they were removed from the LEAF-Q when the validation process found an association only with disordered eating rather than measured LEA [59]. In the present study, we found that adverse dizziness scores were associated with higher cortisol:insulin ratio and lower glucose and insulin. As there was no screening for disordered eating in the current validation, it is not possible to determine whether this was a sign of LEA or DE, and this limitation is acknowledged.

Higher illness scores were associated with lower T_3 among our participants. Although this is in keeping with the findings of studies involving menstrual dysfunction [99], LEAF-Q scores [64] and participation in leanness sports [39], no association between illness and markers of LEA was seen in a large-scale mixed sport cohort [49]. Further research is required to understand the interaction between the immune system and EA in athlete populations. Indeed, a recent review of the complex relationship between nutrition and immune tolerance/resistance has recently proposed that energy restriction per se may not increase illness risk, and that previous associations reported in studies of athletic populations may be mediated by a common co-morbidity such as higher ratings of psychological stress [100]. Indeed, one study has reported an apparent disconnect between EA and the occurrence of upper respiratory infections in athletes who commenced high-intensity interval training [101]. Further research on this theme is warranted.

Other unexpected findings in the present study include the association between poorer wellbeing and recovery ratings and higher total, but not LDL, cholesterol. The reasons for this association are unclear and worthy of further investigation to identify whether this is a repeatable association and the possible underlying mechanisms. Furthermore, athletes in weight sensitive sports were noted to have higher body fat than those from non-weight sensitive sports. It is possible that this is due to perturbations previously observed in some groups assessed as being exposed to LEA [29] or poor within-day energy balance [102].

The clinical indicators most often associated with adverse questionnaire responses in our participants and the differentiation between LEA cases and controls were testosterone, cortisol, insulin, cortisol:insulin ratio, T_3 and RMR. This is supported by other studies on LEA, within-day energy balance or energy restriction in males [14,58,96,97]. These markers may be most helpful in studying LEA in male athlete populations. Raised LDL cholesterol was associated with other clinical markers in the current study, but none fit the pattern expected with LEA. Further investigations of interactions between cholesterol metabolism and LEA or coincidental metabolic impairments are warranted, noting that LDL cholesterol is higher in anorexia nervosa patients than controls [103].

Overall, we found that LEA in a field setting is difficult to characterize with errors of measurement compounded by differences in the presentation of acute and chronic changes in clinical markers and individual differences in presentation [48]. Indeed, while we found an overlap in clinical presentations, there was also a divergence (Table 5) in both the clinical markers and the questions showing perturbations. Our results further highlight the folly of previous approaches to screening for LEA in male populations, including the use of the LEAF-Q from which questions on menstrual function have been excluded [67] or replaced with male reproductive questions [66], or those based on adaptations of female specific questionnaires that have not been validated for males [25,26].

The LEAF-Q was founded on the female athlete triad, associating questions on injury with low BMD, gastrointestinal dysfunction with LEA and the menstrual function score with clinically verified menstrual dysfunction [59]. In the current LEAM-Q validation, however, neither injury nor gastrointestinal symptoms were associated with LEA biomarkers with adequate sensitivity and were excluded from the questionnaire. The lack of utility of questionnaires developed for female populations in male cohorts is not unique to LEA; researchers have identified flaws in the application of female-derived surveys of disordered eating and body image [104–106] and have noted erroneous outcomes in clinical and research activities in other areas due to the use of poor screening tools [107].

The inclusion of the sex-drive variable in the updated version of the LEAM-Q warrants several comments. It was included as a proxy marker of reproductive function, to mimic questions around the menstrual cycle included in the LEAF-Q. It was not included in the first version of the LEAM-Q, due to external advice that it is challenging to obtain accurate information on sex drive given the possibility of stigma or embarrassment around admitting low sex drive or reduced morning erections. Furthermore, the accuracy of self-reports of sex drive has not been established. Nevertheless, subsequent discussion among the research team considering growing recognition of endocrine changes in male athletes associated with LEA [3,108] increased our interest in collecting information on sex drive within the LEAM-Q. Despite the caveats around such self-reported information, accuracy of recall over the last month, and the relatively smaller sample size in the analysis of this factor, we found perturbations to sex drive to be the most consistent indicator of LEA in male athletes, being the only questionnaire metric that differed between cases and controls. Further investigation is warranted in both males and females; indeed, it may be useful to interrogate sex drive in female populations as an adjunct to information on menstrual function or to address situations where the use of hormonal contraceptives interferes with an assessment of menstrual status. Indeed, females with anorexia nervosa are reported to experience a lower sex drive [109].

We were deliberate in designing our study to investigate a collection of biomarkers of LEA rather than assessing EA in each participant based on information on energy intake, exercise energy expenditure and FFM. We note both the lack of a standard methodology for EA assessment and the errors involved in estimating each of these components [48]. These issues, as well as the disconnect between an acute assessment and chronic time-course, over which an energy mismatch might have occurred, explain the conflicting outcomes of EA assessments and biomarkers of LEA in many studies [110]. No single marker is successful in identifying LEA; exposure may be best identified from a cluster of symptoms and with the exclusion of a differential diagnosis for some factors [111,112]. For example,

Rogers and colleagues found that while 80% of an athlete cohort showed one or more of the possible symptoms associated with RED-S, only 11% recorded a low RMR [63]. Meanwhile, Stenqvist et al. identified male athletes with low RMR in the absence of any markers of LEA, including effects on BMD [97].

Although the best possible effort was made to characterise the clinical markers identifying LEA in the present study, further research is required to better identify thresholds indicative of perturbation in male athletes. In this study, the lowest or highest quartile was used for several variables where sub-clinical deficiency is likely to be important, but reference ranges for the marker are not yet available. Consistency in these cut-points will be important for future research and it is encouraging to see this develop for testosterone [5]. Ratios of cortisol:insulin and free testosterone:cortisol were significantly different between LEA cases and controls in our study; however, inconsistency of measurement units in previous research makes comparisons or the development of normative ranges challenging. While the overall data set was relatively large, key variables such as insulin, testosterone, cortisol and sex drive questions were only included in version 2 of the study and, as such, the sample size is much smaller for these key areas.

Previous research has shown that male athletes with higher exercise energy expenditure have lower EA [113] and males with eating disorders are more likely to have a focus on exercise rather than diet as a weight loss strategy [93]. Questions around training load and intensity have been successful in identifying male athletes with low testosterone [68] and exercise dependence with low testosterone cortisol ratio and high cortisol insulin ratio [24]. The LEAM-Q included a question on training hours, which was associated with aspects of sex drive. Given the diversity of the sports included in this investigation, this question was inadequate to capture differences in training load and the further development of questions of this nature may be worthwhile and have been included in the amended version of the LEAM-Q questionnaire.

A possible limitation of the current study was that, by nature, the multicentre, multicountry data collection resulted in multiple DXA machines, technicians and reference populations being used for assessment. Similarly, RMR was measured variously by a first principles and metabolic cart method and blood analysis was undertaken by multiple laboratories. Whilst these differences are acknowledged, the potential impact was minimised by using best practice protocols for data collection and using the lowest quartile for the testing site at which it was collected. Furthermore, the small differences in estimates of FFM and subsequent interpretation of RMR would likely be negligible.

The difficulty in validating this screening questionnaire may be due, in part, to the difficulty of identifying LEA in males and/or the need for further development of target questions. The specificity of key issues within certain sports or events is also recognised, meaning that although a questionnaire may successfully identify risk factors in a homogenous group, it may be less sensitive or play an alternative role in a different group or mixed population. For example, Rogers et al. found that the LEAF-Q, validated in endurance and weight-sensitive athletes, was able to "rule out" those at low risk of LEA in a mixed population of female athletes, while those scoring above the designated threshold would require further clinical assessment to identify LEA [96]. Indeed, while sex drive successfully differentiated between LEA cases and controls in the current study, it has also been used as a proxy for EHMC [68,70] and for disordered eating and exercise dependence [66]. Whilst these conditions are interrelated, a screening questionnaire can only act as a flag for further clinical assessment and not for diagnosis. It is noted that perturbations in testosterone and sex drive have been considered markers for EHMC, but in this study they were also associated with other endocrine and metabolic perturbations, highlighting the need for clarification of the interplay between LEA and EHMC.

This study provides unique information on the expression of LEA in a large group of male athletes across a range of sports and highlights the importance of asking about sex drive when screening male athletes for RED-S. It also confirms the need for sex-specific, sport-specific and, perhaps, calibre-specific screening tools in athlete populations. The

LEAM-Q developed for the current study failed to clearly distinguish between athletes considered to be LEA cases and their control counterparts, with only the sex-drive subsection having this utility. Nevertheless, it provides a bank of content-validated questions that could be of use for future studies in different populations. Further work from our group will focus on a new version of the questionnaire that extends the investigation of sex drive, with the addition of information on flux of body mass/composition and training load.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu14091873/s1, File S1: LEAM-Q questionnaire and scoring tool original. File S2: LEAM-Q questionnaire and scoring tool, final.

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LEAM Q -

A questionnaire for male athletes

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The low energy availability in males questionnaire (LEAM –Q), focuses on physiological symptoms of relative energy deficiency. The following pages contain questions regarding health, injuries, cold sensitivity, gastrointestinal function and recovery. We appreciate you taking the time to fill out the LEAM-Q and the results will be treated as confidential.

Name: _____

Address: _____

_____

E-mail: _____

Cell phone: _____

Sport: _____

- How old were you when you began to specialize in your sport?:_____ age
- What level of athlete are you?
  Club ☐

  National team ☐

  Professional ☐

  Other ☐
- Are you a full-time athlete? Yes ☐ No ☐
- If not, what occupation do you have beside your sport?
  Full time job ☐

  Part time job ☐

  Student ☐

  Other ☐
- What is your maximal oxygen consumption (Vo_2max)?

  _____ml/kg/min or _____l/min

  I do not know/I have never measured it ☐

- Your best results at World Championship, Olympic Games or World Cup?
  1st to 3rd place □
  4th to 6th place □
  7th to 10th place □
  11th place or lower □
  I have never competed at this level □
  I don't remember □

- Your normal amount of training in the preparation or basic period (not competition) on average per month:

  _____ hours/month

- Age: _____(years)
- Height: _____(cm)
- Present weight: _____(kg)
- Your highest weight with your present height: _____ (kg)
- Your lowest weight with your present height: _____ (kg)
- What is your preferred body weight during competition? _____ (kg)
- What is your body fat percentage (if it has been measured)? _____ (%)
- Chronic illness (e.g. diabetes, Crohn's Disease)?
  Yes □ No □

  If yes, which one (s)? _____

- Food allergy or intolerance (e.g. nut allergy, celiac disease, lactose intolerance)?
  Yes □ No □

  If yes, which one (s)? _____

1. Dizziness

Mark the response that most accurately describes your situation

A: Do you feel dizzy or lightheaded when you rise quickly?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B: Do you experience problems with vision (blurring, seeing spots, tunnel vision, etc.)

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

2. Gastrointestinal function

A: Do you feel gaseous or bloated in the abdomen?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B: Do you get cramps or stomach ache?

☐ Yes, several times a day ☐ Yes, several times a week

☐ Yes, once or twice a week or more seldom ☐ Rarely or never

C: How often do you have bowel movements on average?

☐ Several times a day ☐ once a day ☐ Every second day ☐ Twice a week
☐ Once a week or more rarely

D: **How would you describe your normal stool?**

☐ Normal (soft) ☐ Diarrhoea-like (watery) ☐ Hard and dry

Comments regarding gastrointestinal function: _____

3. Regulation of body temperature at rest

A: Are **you very cold even when you are normally dressed?**

☐ Yes, almost every day ☐ Several times a week

☐ Once or twice a week or more seldom ☐ Rarely or never

B: Do you dress more warmly than your companions regardless of the weather?

☐ Yes, almost always ☐ Yes, sometimes ☐ Rarely or never

4. Health problem interfering with training or competition plans

Mark the response that most accurately describes your situation

In the following we will ask you some question regarding how often, during the last 6 month you have had to change plans concerning training or competition or not been able to perform your maximal during training due to a sport injury or illness. An *acute injury* appears suddenly for an obvious reason at a specific time (e.g. a sprain). An injury due to *overload* develops gradually (e.g. shin or Achilles, stress fracture).

A: How many acute injuries have you had during the past 6 months?

_____ acute injuries.

B: How many overload injuries (the same reoccurring overload injury, counts as a new injury for every new period) have you had during the past 6 months?

_____ overload injuries.

C. How many breaks in training have you had due to illness during the past 6 months?

_____ breaks in training due to illness.

D. During the last 6 months, how many days in a row, <u>at the most</u>, have you been absent from training/competition <u>o</u>r not been able to perform <u>optimally</u> at training/competition due to an injury (acute/overload) or illness?

	None	1-7 days	8-14 days	15-21 days	≥ 22 days
Acute injury	☐	☐	☐	☐	☐
Overload injury	☐	☐	☐	☐	☐
Illness	☐	☐	☐	☐	☐

Comments concerning your injuries: _____

_____

Comments concerning your illnesses: _____

_____

5. Well-being & Recovery

Mark the response that most accurately describes your situation

A: Fatigue

A:1 I feel tired from work/school

☐ Yes, several times a day ☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

A:2 I feel overtired

☐ Yes, several times a day ☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

A:3 I'm unable to concentrate well

☐ Yes, several times a day ☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

A:4 I feel lethargic

☐ Yes, several times a day ☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

A:5 I put off making decisions

☐ Yes, always ☐ Yes, often ☐ Yes, sometimes ☐ Rarely or never

---

B: Fitness

B:1 Parts of my body are aching

☐ Yes, several times a day ☐ Yes, several times a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B:2 My muscle feels stiff or tense during training

☐ Yes, almost every training session ☐ Yes, often ☐ Yes, sometimes ☐ Rarely or never

---

B:3 I have muscle pain after performance

☐ Yes, after almost every training session ☐ Yes, often ☐ Yes, sometimes ☐ Rarely or never

---

B:4 I feel vulnerable to injuries

☐ Yes, always ☐ Yes, in most training periods ☐ Yes, in some training periods ☐ Rarely or never

---

B:5 I have a headache

☐ Yes, almost daily ☐ Yes, several days a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B:6 I feel physically exhausted

☐ Yes, almost daily ☐ Yes, several days a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

B:7 I feel strong and am making good progress with my strength training

☐ Yes, always ☐ Yes, in most training periods ☐ Yes, in some training periods ☐ Rarely or never

5. Continued

Mark the response that most accurately describes your situation

C: Sleep

C:1 I get enough sleep

☐ Yes, almost every night ☐ Yes, several nights a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

C:2 I fall asleep satisfied and relaxed

☐ Yes, almost every night ☐ Yes, several nights a week ☐ Yes, once or twice a week or more seldom ☐ Rarely or never

---

C:3 I wake up well rested

☐ Yes, almost every morning ☐ Yes, several days a week ☐ Yes, once or twice a week or more seldom ☐ Rarely or never

---

C:4I sleep restlessly

☐ Yes, almost every night ☐ Yes, several nights a week ☐ Yes, once or twice a week or more seldom ☐ Rarely or never

---

C:5 My sleep is easily interrupted

☐ Yes, almost every night ☐ Yes, several nights a week ☐ Yes, once or twice a week or more seldom ☐ Rarely or never

---

C:6 During the last month, how many hours (mean/night) have you slept (this can be different from the number of hours that you have spent in bed) Sleep (hours) per night:

_____Hours

D: Recovery

D:1 I recover well physically

☐ Yes, after almost all training sessions ☐ Yes, often ☐ Yes, sometimes
☐ Rarely or never

D:2 I'm in good physical shape

☐ Yes, always ☐ Yes, mostly ☐ Yes, sometimes ☐ Rarely or never

---

D:3 I feel I'm achieving the progress in training and competition that I deserve

☐ Yes, always ☐ Yes, in most training periods ☐ Yes, in some training periods ☐ Rarely or never

---

D:4 My body feels strong

☐ Yes, almost every day ☐ Yes, several days a week
☐ Yes, once or twice a week or more seldom ☐ Rarely or never

Energy Levels

E:1 I feel very energetic in general

□ Yes, almost every day □ Yes, several days a week

□ Yes, once or twice a week or more seldom □ Rarely or never

E:2 I feel invigorated for training sessions and ready to perform well

□ Yes, almost every day □ Yes, several days a week

□ Yes, once or twice a week or more seldom □ Rarely or never

E-3 I feel happy and on top of my life outside sport

□ Yes, almost every day □ Yes, several days a week

□ Yes, once or twice a week or more seldom □ Rarely or never

E-4 I feel down and less happy than I used to feel or would like to feel

□ Yes, almost every day □ Yes, several days a week

□ Yes, once or twice a week or more seldom □ Rarely or never

Sex drive

F:1 Your sex drive can be a marker of the balance between training, rest and nutrition.

a) In general I would rate my sex drive as

□ high □ moderate □ low □ I don't have much interest in sex

b) Over the last month I would rate my sex drive as

□ stronger than usual □ about the same as usual □ a little less than usual

□ much less than usual

F:2 It is common to wake in the morning with an erection

a) Over the last month, has this happened

□ 5-7 per week □ 3-4 a week □ 1-2 a week □ Rarely or never

b) Compared to what you would consider is normal for you is this

□ More often □ about the same □ a little less often □ much less often

Thank you!

LEAM Q -

Scoring Key

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1 A: Do you feel dizzy when you rise quickly?
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never

1 B: Do you experience problems with vision (blurring, seeing spots, tunnel vision, etc.)
3 Yes, several times a day 2 Yes, several times a week 1 Yes, once or twice a week or more seldom 0 Rarely or never

2 A: Do you feel gaseous or bloated in the abdomen?
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never

2 B: Do you get cramps or stomach ache?
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never

2 C: How often do you have bowel movements on average?
1 Several times a day, 0 once a day, 2 Every second day, 3 Twice a week, 4 Once a week or more rarely

2 D: **How would you describe your normal stool?**
0 Normal (soft), 1 Diarrhoea-like (watery), 2 Hard and dry

3 A: Are you very cold even when you are normally dressed?
3 Yes, almost every day, 2 Several times a week, 1 Once or twice a week or more seldom, 0 Rarely or never

3B: Do you dress more warmly than your companions regardless of the weather?
3 yes, almost always 1 Yes, sometimes 0 rarely or never

4 A: How many acute injuries have you had during the past 6 months?
The number of acute injuries is the score

4 B: How many overload injuries (the same reoccurring overload injury, counts as a new injury for every new period) have you had during the past 6 months?
The number of overload injuries is the score

4 C. How many pauses in training have you had due to illness during the past months?
The number of pauses in training due to illness is the score

4 D. During the last 6 months, how many days in a row, <u>at the most</u>, have you been absent from training/competition <u>or</u> not been able to perform <u>optimally</u> at training/competition due to an injury (acute/overload) or illness?

	Non	1-7 days	8-14 days	15-21 days	More than 22 days
Acute injury	0	1	2	3	4
Overload injury	0	1	2	3	4
Illness	0	1	2	3	4

5 A:1 I feel tired from work/school
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom, 0 Rarely or never

5 A:2 I feel overtired
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never

5 A:3 I'm unable to concentrate well
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom, 0 Rarely or never
5 A:4 I feel lethargic
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom, 0 Rarely or never
5 A:5 I put off making decisions
3 Yes, always 2 Yes, often 1 Yes, sometimes 0 Rarely or never
5 B:1 Parts of my body are aching
3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never
5 B:2 My muscles feel stiff or tense during training
3 Yes, almost every training session, 2 Yes, often, 1 Yes, sometimes, 0 Rarely or never
5 B:3 I have muscle pain after performance
3 Yes, after almost every training session, 2 Yes, often, 1 Yes, sometimes, 0 Rarely or never
5 B:4 I feel vulnerable to injuries
3 Yes, always, 2 Yes, in most training periods, 1 Yes, in some training periods, 0 Rarely or never
5 B:5 I have a headache
3 Yes, almost daily, 2 Yes, several days a week, 1 Yes, once or twice a week or more seldom, 0 Rarely or never
5 B:6 I feel physically exhausted
3 Yes, almost daily, 2 Yes, several days a week, 1 Yes, once or twice a week or more seldom, 0 Rarely or never
5 B:7 I feel strong and am making good progress with my strength training
0 Yes, always 1 Yes, in most training periods 2 Yes, in some training periods 3 Rarely or never
5 C:1 I get enough sleep
0 Yes, almost every night, 1 Yes, several nights a week, 2 Yes, once or twice a week or more seldom, 3 Rarely or never
5 C:2 I fall asleep satisfied and relaxed
0 Yes, almost every night, 1 Yes, several nights a week, 2 Yes, once or twice a week or more seldom, 3 Rarely or never
5 C:3 I wake up and well rested
0 Yes, almost every morning, 1 Yes, several days a week, 2 Yes, once or twice a week or more seldom 3 Rarely or never
5 C:4 I sleep restlessly
3 Yes, almost every night, 2 Yes, several nights a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never
5 C:5 My sleep is easily interrupted
3 Yes, almost every night, 2 Yes, several nights a week, 1 Yes, once or twice a week or more seldom 0 Rarely or never
5 D:1 I recover well physically
0 Yes, after almost all training sessions, 1 Yes, often, 2 Yes, sometimes, 3 Rarely or never
5 D:2 I'm in good physical shape
0 Yes, always, 1 Yes, mostly, 2 Yes, sometimes, 3 Rarely or never

5 D:3 I feel I am achieving the progress in training and competition that I deserve
0 Yes, always, **1** Yes, in most training periods, **2** Yes, in some training periods, **3** Rarely or never
5 D:4 My body feel strong
0 Yes, almost every day, **1** Yes, several days a week, **2** Yes, once or twice a week or more seldom, **3** Rarely or never
5 E:1 I feel very energetic in general
0 Yes, almost every day, **1** Yes, several days a week, **2** Yes, once or twice a week or more seldom, **3** Rarely or never
5 E:2 I feel invigorated for training sessions and ready to perform well
0 Yes, almost every day, **1** Yes, several days a week, **2** Yes, once or twice a week or more seldom, **3** Rarely or never
5 E:3 I feel happy and on top of my life outside sport
0 Yes, almost every day, **1** Yes, several days a week, **2** Yes, once or twice a week or more seldom, **3** Rarely or never
5 E:4 I feel down and less happy than I used to feel or would like to feel
3 Yes, almost every day, **2** Yes, several days a week, **1** Yes, once or twice a week or more seldom, **0** Rarely or never
5 F:1a I would rate my sex drive as
0 high, **1** moderate, **2** low, **3** I don't have much interest in sex
5 F:1b over the last month I would rate my sex drive as
0 stronger than usual, **0** about the same, **1** a little less than usual **2** much less than usual
5 F:2a Morning erections: over the last month this has happened
0 5-7 per week, **0** 3-4 a week, **1** 1-2 a week, **2** rarely or never
5 F:2b compared to what you would consider normal for you is this
0 more often, **0** about the same, **1** a little less often, **2** much less often

LEAM Q -
A questionnaire for male athletes

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The low energy availability in males questionnaire (LEAM –Q), focuses on physiological symptoms of relative energy deficiency. We appreciate you taking the time to fill out the LEAM-Q and the results will be treated as confidential.

Name: _____

E-mail: _____

Phone: _____

Sport: _____

- Age: _____(years)
- How old were you when you began to specialize in your sport?_____ Age
- Height: _____(cm)
- Present weight: _____(kg)
- Your highest weight with your present height: _____ (kg)
- Your lowest weight with your present height: _____ (kg)
- What is your preferred body weight during competition? _____ (kg)
- What is your body fat percentage (if it has been measured)? _____ (%)
- Do you currently diet or restrict your food intake in order to make weight or achieve your body composition goals? Choose the answer that best suits your situation

  No, I eat as much as I like/need most of the time □

  Yes, I watch what I eat but I can still eat freely □

  Yes, I am actively trying to lose weight/body fat to achieve a target □

  Yes, I restrict what I eat most of the time to manage my body weight/composition □

- What level of athlete are you?
  Club □

  National team □

  Professional □

  Other □

- Are you a full-time athlete? Yes □ No □

- If not, what occupation do you have beside your sport?
  Full time job □

  Part time job □

  Student □

  Other □

- What is your maximal oxygen consumption (Vo_2max)?

  _____ml/kg/min or _____l/min

  I do not know/I have never measured it □

- Your best results at World Championship, Olympic Games or World Cup?
  1st to 3rd place □

  4th to 6th place □

  7th to 10th place □

  11th place or lower □

  I have never competed at this level □

  I don't remember □

- Your normal amount of training in the preparation or basic period (not competition) on average per week:

  _____ hours/week

  Of this training time, roughly what percentage would you spend working at

  _____ Low intensity (<35% VO_{2max})

  _____ medium intensity (35-75% VO_{2max})

  _____ high intensity (>70% VO_{2max})

- In your general life or work outside of prescribed training for your sport, would you describe your activity level as

  Low
  (low activity outside of formal training) □

  Medium
  (social sport, short commute) □

  High
  (physical job, long commute) □

Sex Drive

Mark the response that most accurately describes your situation

A: Your sex drive can be a marker of the balance between training, rest and nutrition.

b) In general I would rate my sex drive as

□ high □ moderate □ low □ I don't have much interest in sex

2. Over the last month I would rate my sex drive as

□ stronger than usual □ about the same as usual □ a little less than usual

□ much less than usual

B: It is common to wake in the morning with an erection

1. Over the last month, has this happened

□ 5-7 per week □ 3-4 a week □ 1-2 a week □ Rarely or never

Thank you!

LEAM Q -

Scoring Key

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Sex Drive

A:1 I would rate my sex drive as

0 high, **1** moderate, **2** low, **3** I don't have much interest in sex

A:2 over the last month I would rate my sex drive as

0 stronger than usual, **0** about the same, **1** a little less than usual **2** much less than usual

B:1 Morning erections: over the last month this has happened

0 5-7 per week, **0** 3-4 a week, **1** 1-2 a week, **2** rarely or never

B:2 Compared to what you would consider normal for you is this

0 more often, **0** about the same, **1** a little less often, **2** much less often

Low sex drive is identified when

2 or more is scored on A1 OR

2 or more is scored on B1 AND 1 or more on B2

7. Interlinking chapter

In Study 2 a validation process was undertaken for a questionnaire developed to screen for LEA in male athletes. Despite a successful test-retest process, several significant associations between clinical variables and questionnaire responses found in the multivariate analysis and sufficient sensitivity determined by the ROC analysis the resulting questionnaire was unable to distinguish between LEA cases and controls with the total questionnaire score or any subsection except sex drive. A final questionnaire was proposed including sex drive and additional questions regarding weigh flux, diet restriction and training load. The differences between the questions of importance for male and female athletes are noted and highlight the flaws in the current research literature where questionnaires that have not been validated in male populations are being used for this purpose.

Study 3 was designed to look at possible avenues, outside of LEA, for negative changes to bone health. This study investigated the impact of pre-exercise calcium intake on bone turnover markers over a typical training day involving two endurance training sessions.

8. The impact of acute calcium intake on bone turnover markers during a training day in elite male rowers

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9. Discussion & Conclusions

The reduction of bone stress injuries in sport is an important theme in sports medicine research and practice. Long term changes to bone mineral density and acute responses of bone turnover markers are influenced by nutrition and present a potential opportunity to reduce injury risk. Due to the lack of research on factors associated with bone injury in rowing, this thesis aimed to address the gaps in the literature by investigating

i. Factors associated with rib stress injury history including diet restriction, menstrual dysfunction, training age, calcium intake and vitamin D and K status.
ii. Validation of a screening tool LEA in male elite athletes (LEAM-Q)
iii. The impact of pre-exercise calcium intake on markers of bone turnover in elite male rowers

The findings of these studies will be outlined in detail below. Together, they demonstrate that BMD and RSI history are associated with diet restriction in both males and females. Menstrual dysfunction is associated with RSI in females and utilising questions relating to sex drive from the LEAM-Q may provide further capacity to understand this relationship in males. A typical rowing training day causes prolonged periods of raised markers of bone breakdown which are attenuated when calcium is consumed 2 hours pre-exercise. This may be an important strategy in protecting long term bone health in this population. The research undertaken provides a package of potential strategies for injury risk reduction including a focus on adequacy of EA, appropriate monitoring of LEA and BMD, and pre-exercise calcium intake.

9.1 Novel Findings

A high volume of training is needed to achieve and maintain the necessary physiological and physical attributes of high-performance rowers. Rowers have been identified as a high-risk group for bone-related injuries with rib stress injuries being the highest burden injury, occurring in 16% of an elite rowing population over two Olympiads and causing one in five of the lost training days experienced by rowers (Trease et al. 2020). Harris et al. identified that athletes with a rib stress injury during the Rio Olympic cycle failed to win an international medal in the year of their injury or at the Olympic Games (Harris et al. 2020). Injury prevention is a key focus shared by athletes, coaches and support staff within rowing programmes. Surprisingly, although rib stress injury represents the greatest time loss burden within rowing, the potential contributors, including nutrition-associated factors, have been poorly described, with only two small sample studies being available (Vinther et al. 2005, Baker et al. 2022). *Study one* was the first investigation of bone issues in high performance rowers to adequately represent both sex and weight category. The key finding of this research was that BMD, either AP spine, proximal femur or rib were associated with history of RSI. In addition, diet

restriction, higher body fat and menstrual function disturbances showed association with RSI. Rib BMD was associated with sex, weight category, age and diet restriction. Age and diet restriction were associated with spine BMD, while femur BMD was associated with sex, weight category and history of RSI.

The sports medicine literature frequently notes the association of BMD and bone stress injuries in athletes (Bennell et al. 1996b, Abbott et al. 2020), including rowing populations (Vinther et al. 2005, Dimitriou et al. 2014). The findings of the current study, in which rib, spine and femur BMD were similarly associated with RSI history, support this relationship. It is of interest that, despite this association, the BMD of the cohort was within the standards considered to be "healthy", even when following the recommendations of sports medicine expert panels to define normal bone health in athletes from a Z-score ≥-1.0, as opposed to ≥ -2.0 in a young non-athlete population (Mountjoy et al. 2015, De Souza et al. 2017, International Society for Clinical Densitometry 2019, Fredericson et al. 2021). It is possible that the highly specific and repetitive loading associated with some sports results in BMD that is higher than seen in the general population but still insufficient to meet the demands of the sport (Jonvik et al. 2022). Alternatively, it is acknowledged that BMD may not reflect the microarchitecture of the bone or other characteristics that are needed to provide adequate resilience. In any case, these findings support the call for the development of sports-specific, site-specific guidelines for BMD that consider the characteristics and requirements of the sport and allow for earlier detection of sub-optimal bone health (Moran et al. 2012, Jonvik et al. 2022).

A limitation of this study was the lack of a sedentary group for comparison of BMD which may have provide insights into the benefits or otherwise of high-level rowing training. This need is partly alleviated by the use of Z scores which compared BMD to age, ethnicity and sex matched populations and also through the investigation of BMD and training age. In the current research spine BMD and Z-Score increased with training age but, contrary to expectation, there was a decrease in rib BMD. The reasons for these changes are unclear. Kurgan et al monitored BMD over the course of a training season in female rowers preparing for the Olympic games and noted no change. Z scores were not reported and no comment was made on initial bone health (Kurgan et al. 2018). Similarly, stable BMD was reported in a longitudinal study of elite male rowers (Jurimae et al. 2006), while Masters Level rowers have been shown to have higher BMD than their sedentary counterparts (Sliwicka et al. 2015). Whether rowing training has a beneficial, neutral or negative effect on BMD requires further investigation.

The identification of the utility of rib BMD, assessed from a DXA scan of whole-body composition, as a potential indicator of rib stress risk has important practical findings for the clinician. Such body composition assessments are becoming more widespread in high performance sport, and typically

incur both a lower radiation dose and less requirement for a medical referral than site specific scans of BMD. If this information was already available via separate analysis of pre-existing or scheduled scans, it could be used as a tool for earlier identification of rib stress risk in susceptible rowers. Although Z-scores for such assessments are not available, rowers showing a progressive decline in rib BMD or values below that of their counterparts could be selected for specific bone health assessment and management. It is also possible that serial monitoring of rib BMD might provide an opportunity for secondary prevention (early detection) programs to investigate early interventions designed to improve rib BMD, such as protocols for chest wall loading, and provide new opportunities to research reduction in rib injury occurrence.

Calcium and vitamin D are the most cited nutrients influencing bone health in the general population. In contrast to previous research (Tenforde et al. 2010, Moran et al. 2012, Abbott et al. 2020), we failed to find an association between calcium intake or vitamin D status and either BMD or RSI in the current cohort of rowers, possibly because the status was normal for the majority of participants. Although the study by Baker et al (Baker et al. 2022) reported differences in calcium intakes between injured and uninjured rowers, the small size of the study population may have introduced an unknown bias. Nevertheless, given the widely accepted impact of calcium intake and vitamin D status on BMD (Ebeling et al. 2021), it seems wise for the practitioner to monitor these to rule out sub-optimal intake or deficiencies. Vitamin K status was normal across the group and there is insufficient support from the literature to recommend routine monitoring (Braam et al. 2003, Fang et al. 2012) . Vitamin K status was similarly normal and no relationship was seen with either RSI or BMD.

Low energy availability has been associated with low BMD and bone stress injuries across a range of athlete populations (Heikura et al. 2018b). A limitation of the current study was the absence of a direct measurement of energy availability, but rather a graded self-report of diet restriction and menstrual dysfunction. While this was a relatively crude method of assessment, associations were seen with both rib and spine BMD, with greater levels of restriction being associated with lower BMD and RSI history. These questions were designed to suit the unique perspective of the group around these issues and could be easily applied in a clinical setting as a simple monitoring method. It is possible that diet restriction represents a collider bias where lower energy intake provides a lower level of other key nutrients for bone health that are ultimately responsible for the differences seen. However, given the lack of association with calcium, vitamin D and K, this seems less likely.

Increased severity of diet restriction was associated with stepwise reduction in rib and spine BMD for males but not for females. This may be a factor of the specific study population rather than a sex difference *per se.* For example, the lightweight male participants in this cohort were less likely to

access the available nutrition support and were typically less likely to be 'natural' lightweights (i.e. their habitual body mass was higher than the weight limits and required significant manipulation prior to competition weigh-in). If rib BMD is to be used as part of athlete monitoring any sex-related differences will need to be clarified. The association of RSI with higher body fat levels is likely to be an indirect relationship, reflecting either suppressed metabolism following restriction of EA (VanHeest et al. 2007) or the lower training status or individuals within the cohort. The study population consisted of those selected into, or in contention for, international standard crews. However, the more inexperienced rowers, such as those selected to their first team or those selected to gain experience, rather than win medals, may not have the same body composition as a more experienced rower and may be at more risk of RSI when progressing to a higher training load.

Anthropometric features such as the proportion between upper and lower body lean mass, arm span and sitting height were also investigated. This followed early research (Vinther et al. 2006) suggesting movement patterns and imbalances between upper and lower body strength may be important in the development of RSI. Of all the anthropometric measures arm span was the only measure associated with RSI with greater arm span associated with increased history of RSI in models including spine and rib BMD. As arm span is not changeable and did not add strength to the models it was not pursued further though it may be of interest to note. Finally, the case series of participants with a history of multiple injuries suggests that diet restriction, menstrual history, vitamin D status and changes in training load may be important contributors or indictors of the risk of repetitive injuries. Interestingly, there was no difference in menarche or current menstrual status seen for female rowers with single RSI history.

Study two followed the theme of diet restriction, aiming to validate a screening tool for energy availability for male athletes. Specific tools currently exist for female athletes but not for males. Despite widespread interest, this was the first large scale attempt to validate a specific LEA screening tool for male athletes. Associations were seen between the LEAM-Q questions and clinical markers of LEA with adequate sensitivity in areas of dizziness, illness, wellbeing and fatigue and sex drive. Apart from sex drive, however, the battery of questions in the LEAM-Q was unable to robustly distinguish between LEA cases or controls, as categorised by the researchers, for total score or any sub-score. This is an important finding given the number of questionnaires currently used to identify LEA in male athletes that are either validated only in females or not validated at all. Those classified as having low sex drive by the LEAM-Q questionnaire demonstrated multiple perturbations in clinical markers of LEA. A secondary finding was that perturbations in clinical markers of LEA tended to 'cluster' but did not present uniformly across cases. The presentation of male athletes with LEA was different to characteristics shown in the literature on female athletes with LEA, both in the pattern of the questionnaire responses and the clinical markers.

Responses to the LEAM-Q questionnaire found several associations between sub-scores and perturbations in individual clinical markers. For example, sex drive was associated with total testosterone, T3, insulin, free testosterone:cortisol ratio while weight flux was associated with cortisol:insulin ratio, dizziness was associated with glucose and insulin and insulin:cortisol ratio, illness was associated with T3, and wellbeing and fatigue were associated with high total cholesterol. Unlike the LEAF-Q for female athletes, which found an association between gastrointestinal symptoms and characterized LEA (Melin et al. 2014), the male participants categorised as cases in the present study did not have higher gastrointestinal scores than controls. Nevertheless, participants with low T3 and low spine Z-score did record higher scores on the gastrointestinal sub-section, the physiological basis for which is unclear. Gastrointestinal symptoms have been previously associated with self-reported exercise dependence and disordered eating scores in male athletes (Kuikman et al. 2021), and in male eating disorder populations (Silla et al. 2021). Although there is a possibility of a sex-difference, gastrointestinal symptoms may have a greater link with the athlete's specific sporting activities. Indeed, a cohort of female athletes from mixed range of sports failed to show the association between gastrointestinal symptoms and LEA (Rogers et al. 2021b) previously reported in the LEAF-Q validation undertaken in endurance and weight sensitive sports (Melin et al. 2014).

Our study failed to show an association between clinical variables and questions around sleep or thermoregulation, and further research on these themes seems less likely to be productive. Although injury scores were associated with several of our biomarkers of LEA, the sensitivity of these scores was low. Indeed, unlike the LEAF-Q validation and other studies in female athletes (Melin et al. 2014, Rauh et al. 2014), our study failed to find an association between injury scores and BMD (Kuikman et al. 2021). Typically, studies in both male and female endurance athletes have found correlations between bone stress injury rates and BMD (Heikura et al. 2018b), with one investigation of male athletes reporting that a cumulative risk assessment score incorporating both LEA and BMD (Kraus et al. 2019) was predictive for bone stress injuries (Kraus et al. 2019). However, we note the lack of association between LEA and injury in a large scale, mixed sport female population (Ackerman et al. 2019) and suggest that in studies involving a diversity of sports, such as the present investigation, injury causation is likely to be multifactorial and less tightly related to LEA. It is possible that greater targeting of questions around specific injury risks within a uniform athlete group may improve the sensitivity of this factor in predicting LEA, but this would also reduce the applicability of the questionnaire across sports; this has been noted for the LEAF-Q (Rogers et al. 2021b). Failure to find relationships between BMD, LEAM-Q questions and other markers of LEA in the current cohort may be due to the disassociation between acute measurements and the chronic nature of bone health (Hooper et al. 2017, Stenqvist et al. 2021).

Questions around dizziness were included in the LEAM-Q battery although they were removed from the LEAF-Q when the validation process found an association only with disordered eating rather than measured LEA (Melin et al. 2014). In the present study, we found that adverse dizziness scores were associated with higher cortisol:insulin ratio and lower glucose and insulin. As there was no screening for disordered eating in the current validation, it is not possible to determine whether this was a sign of LEA or DE; this limitation is acknowledged.

Higher illness scores were associated with lower T3 among our participants. Although this is in keeping with the findings of studies involving menstrual dysfunction (Shimizu et al. 2012), LEAF-Q scores (Drew et al. 2017b) and participation in leanness sports (Hagmar et al. 2013), no association between illness and markers of LEA was seen in a large-scale mixed sport cohort (Ackerman et al. 2019). Other unexpected findings in the present study include the association between poorer wellbeing and recovery ratings and higher total, but not LDL, cholesterol. The reasons for this association are unclear and merit further investigation to identify whether the association is robust and can be explained.

The clinical indicators most often associated with adverse questionnaire responses in our participants, and the differentiation between LEA cases and controls, were testosterone, cortisol, insulin, cortisol:insulin ratio, T3 and RMR. These findings are supported by other studies on LEA, within day energy balance or energy restriction in males (Koehler et al. 2016, Hooper et al. 2017, Torstveit et al. 2018, Stenqvist et al. 2021). These markers may be most helpful in studying LEA in male athlete populations. Raised LDL cholesterol was associated with other clinical markers in the current study, but none fitting the pattern expected with LEA. Further investigations of interactions between cholesterol metabolism and LEA or coincidental metabolic impairments are warranted, noting that LDL cholesterol is higher in patients with anorexia nervosa patients than controls (Stone 1994).

Overall, we found that LEA in a field setting is difficult to characterize with errors of measurement compounded by differences in the presentation of acute and chronic changes of clinical markers and individual differences in presentation (Burke et al. 2018b). Indeed, while we found overlap in clinical presentations, there was also divergence in both the clinical markers and the questions showing perturbations. Our results further highlight the folly of previous approaches to screening for LEA in male populations, including the use of the LEAF-Q from which questions on menstrual function have been excluded (Slater 2015) or replaced with male reproductive questions (Kuikman et al. 2021) or those based on adaptations of female specific questionnaires that have not been validated in males (Kraus et al. 2019, Keay et al. 2020).

The LEAF-Q was founded on the female athlete triad, associating questions on injury with low BMD, gastrointestinal dysfunction with LEA and the menstrual function score with clinically verified menstrual dysfunction (Melin et al. 2014). In the current LEAM-Q validation, however, neither injury nor gastrointestinal symptoms were associated with LEA biomarkers with adequate sensitivity and were excluded from the questionnaire. The lack of utility of questionnaires developed for female populations in male cohorts is not unique to LEA; researchers have identified flaws in the application of female-derived surveys of disordered eating and body image (Hildebrandt et al. 2010, Mond et al. 2014, Schaefer et al. 2018) and have noted erroneous outcomes in clinical and research activities in other areas due to the use of poor screening tools (Cartagena-Ramos et al. 2018).

The inclusion of the sex-drive variable in the updated version of the LEAM-Q warrants several comments. It was included as a proxy marker of reproductive function, to mimic questions around the menstrual cycle included in the LEAF-Q. It was not included in the first version of the LEAM-Q, due to external advice that it is challenging to obtain accurate information on sex drive given the possibility of stigma or embarrassment around admitting low sex drive or reduced morning erections. Furthermore, the accuracy of self-reports of sex drive has not been established. Nevertheless, subsequent discussion among the research team considering growing recognition of endocrine changes in male athletes associated with LEA (De Souza et al. 2019, Dipla et al. 2021) increased our interest in collecting information on sex drive within the LEAM-Q. Despite the caveats around such self-reported information, and the relatively smaller sample size in the analysis of this factor, we found perturbations to sex drive to be the most consistent indicator of LEA in male athletes, being the only questionnaire metric that differed between cases and controls

We were deliberate in designing our study to investigate a collection of biomarkers of LEA rather than assessing EA in each participant based on information on energy intake, exercise energy expenditure and FFM. We note both the lack of a standard methodology for EA assessment and the errors involved in estimating each of these components (Burke et al. 2018b). These issues, as well as the disconnect between an acute assessment and chronic time-course over which an energy mismatch might have occurred, explain the conflicting outcomes of EA assessments and biomarkers of LEA in many studies (Lane et al. 2021). No single marker is successful in identifying LEA; exposure may be best identified from a cluster of symptoms and with the exclusion of a differential diagnosis for some factors (Logue et al. 2018, Logue et al. 2020). For example, Rogers and colleagues found that while 80% of an athlete cohort showed one or more of the possible symptoms associated with RED-S, only 11% recorded a low RMR (Rogers et al. 2021a). Meanwhile, Stenqvist et al. identified male athletes with low RMR in the absence of any markers of LEA including effects on BMD (Stenqvist et al. 2021).

A possible limitation of the current study was that, by nature, the multicentre, multi-country data collection resulted in multiple DXA machines, technicians and reference populations being used for assessment. Similarly, RMR was measured variously by a first principles method using a bespoke metabolic cart and the more conventional commercial carts, while blood analysis was undertaken by multiple laboratories. Although these differences are acknowledged, the potential impact was minimised by using best practice protocols for data collection and using the lowest quartile for the testing site at which it was collected to identify the outliers. Further, the small differences in estimates of FFM and its contribution to subsequent interpretation of RMR would likely be negligible.

The difficulty in validating this screening questionnaire may be due in part to the difficulty of identifying LEA in males and/or the need for further development of target questions. However, as discussed in reference to findings related to many of the sub-sections, there may be key issues within certain sports or events that are specific to the group. Ultimately, it may be possible to develop screening tools that successfully identify risk factors in a homogenous group but have less sensitivity or play an alternative role in a different group or mixed population. For example, Rogers et al. found that the LEAF-Q, validated in endurance and weight-sensitive athletes, was able to "rule out" those at low risk of LEA in a mixed population of female athletes, while those scoring above the designated threshold would require further clinical assessment to identify LEA (Rogers et al. 2021b). Indeed, while sex drive successfully differentiated between LEA cases and controls in the current study, it has also been used as a proxy for EHMC (Hackney et al. 2017, Logue et al. 2021) and for disordered eating and exercise dependence (Kuikman et al. 2021). Whilst these conditions are interrelated, a screening questionnaire can only act as a flag for further clinical assessment rather than diagnosis. It is noted that perturbations in testosterone and sex drive have been considered markers for EHMC, but in this study they were also associated with other endocrine and metabolic perturbations, highlighting the need for clarification of the interplay between LEA and EHMC.

This study provides unique information into the expression of LEA in a large group of male athletes across a range of sports and highlights the importance of asking about sex drive when screening male athletes for RED-S. It also confirms the need for sex-specific, sport-specific and perhaps calibre-specific screening tools in athlete populations. The LEAM-Q developed for the current study failed to clearly distinguish between athletes considered to be LEA cases and their control counterparts, with only the sex-drive sub-section having this utility. Nevertheless, it provides a bank of content-validated questions that could be of use for future studies in different populations.

Study three investigated a novel nutrition strategy with the potential to support bone health, namely the acute intake of calcium prior to multiple rowing training sessions, representative of a typical training day. This is the first study to investigate the effect of repeated, strenuous, non-weightbearing exercise sessions, undertaken in close succession, on bone turnover markers in elite

athletes. A further novel feature involved the use of dietary protocol to provide gut release of calcium and potentially offset the exercise-associated perturbations to calcium homeostasis. The main findings were: (i) The control trial, involving minimal (<10 mg calcium in the pre-exercise meal) was associated with a drop in serum ionised calcium concentrations with each exercise session. Meanwhile, the intake of a calcium-rich meal (~1000 mg calcium) prior to each session enabled a near maintenance of serum iCa concentrations, with iCa values being higher in the CAL trial than the CON trial for a period of ~7 hours spanning the two training sessions and post-exercise recovery. (ii) The perturbation of iCa in the CON trial was associated with an elevation of serum parathyroid hormone (PTH) and the marker of bone resorption, β-CTX-I, during exercise and recovery, with an apparent accentuation of these changes following the second exercise session. (iii) The effect of a calcium-rich pre-exercise meals in countering exercise-associated reductions in blood calcium concentrations in a single exercise session appears to be repeatable and reduced PTH and β-CTX-I concentrations over a sustained (7 hour) time period. We conclude that athletes who undertake repeated sessions of non-weightbearing activity in close succession daily may be exposed to prolonged periods favoring bone resorption. However, our dietary intervention, shown to be practical to achieve and commensurate with other nutritional goals of elite male athletes, may support bone health by stabilizing the conditions that would otherwise favor bone turnover for a significant portion of the day. While this effect has previously been described in response to a single exercise session (Barry et al. 2011, Haakonssen et al. 2015, Sherk et al. 2017), we now show that it has particular relevance to the 'real-life' training of many competitive athletes.

In this current study cohort, risk of LEA was considered to be low, based on the associated biomarkers measured. Indeed, we found a marginally elevated cortisol as the only abnormality, which was likely explained by the implementation of the study immediately after a short break in the training season. However, if ongoing, elevated cortisol may contribute towards bone loss (Mathis et al. 2013) independent of LEA. Given the importance of rib stress injury to performance, all aspects of bone health support strategies need to be considered.

Bone is a dynamic tissue that is constantly underdoing resorption and formation with the balance between activities contributing towards overall bone health (Dolan et al. 2020). However, previous studies of athletes (Barry et al. 2008, Sherk et al. 2014) have suggested that some exercise activities may create a perturbation in bone turnover favoring resorption, which causes a loss of BMD over time. Exercise of a non-weightbearing nature such as rowing may involve minor stimulus of bone formation via mechanical loading at some sites such as the femur. But even when there is direct loading on bones, such as the rib, it may be insufficient to accrue sufficient BMD or architectural strength to withstand the repetitive forces of training. The current study adds to the growing evidence of a reduction in blood concentrations of the ionized or free calcium, via an unknown

mechanism at the commencement of endurance exercise which triggers a homeostatic response to stabilize blood calcium via an acute PTH-mediated resorption of bone (Bouassida et al. 2003, Barry et al. 2011, Kohrt et al. 2018). Here, CTX-I, released from osteoclasts (proton pump on ruffled border) during the breakdown of collagen fibrils and appearing in the blood stream as β-CTX-I, can be considered an acute marker of bone resorption (Dolan et al. 2020). Previous studies have proposed that this process may expose some athletes to repeated and lengthy periods in which there is elevation of bone resorption (i.e., the duration of their training sessions and the ~2 hours of re-equilibration of markers of bone turnover) (Haakonssen et al. 2015). Our interest in elite rowers, and indeed, the design of this study, draws attention to the high-volume training programs of many endurance athletes in which two or three strenuous exercise sessions are undertaken each day, with subsequent sessions often commencing within the window before apparent restoration of equilibrium of bone turnover to the first workout has occurred. Whilst our study is unable to determine the cumulative impact of these changes over time, it confirmed that each session was associated with a perturbation to blood iCa with downstream effects on PTH and β-CTX-I that suggested an extended (~ 7 h) period of elevated bone resorption. Although we acknowledge that our study relies on relatively acute changes to bone turnover markers, we suggest that these results warrant further investigation of the timing and nature of exercise sessions on bone turnover. Furthermore, we propose that strategies to attenuate the initial perturbation of blood iCa may help to support bone health in these scenarios.

The provision of an alternative source of calcium to buffer exercise-mediated blood calcium losses has been achieved via IV clamps in research settings (Kohrt et al. 2018, Wherry et al. 2021a) as well as the gut release of calcium ingested from supplements (Barry et al. 2011, Sherk et al. 2017) or foods (Haakonssen et al. 2015) in more real world protocols. Here, the timing of intake of calcium appears to be important with studies that have used oral calcium either in close proximity or during exercise showing less clear effects of supplementation, particularly on β-CTX-I (Barry et al. 2011, Sherk et al. 2017). The optimal dose of pre-exercise calcium has not been identified and future investigation should target this issue. Nevertheless, previous work from our group found that the intake of a calcium-rich (1200 mg) meal, consumed 2 hours prior to a cycling session, represented a protocol that integrated gastrointestinal comfort (Haakonssen et al. 2014), pre-exercise fuel goals, and an effective dietary source of calcium to address the exercise-mediated changes to iCa (Haakonssen et al. 2015). The current study confirmed that everyday foods can provide a practical intake of 1000 mg of calcium while simultaneously achieving energy, macronutrient and micronutrient goals for this athletic population. The menu, based on dairy foods, was easily-consumed and could be adapted for an individual with lactose-intolerance, although not suited to vegan eaters. The timing and size of the meal contributed to the fuel goals for each session as well as

daily energy requirements, and our anecdotal observations of good gastrointestinal tolerance are supported by previous systematic measurements of gastrointestinal comfort when a similar meal was consumed prior to a sustained high-intensity exercise time trial (Haakonssen et al. 2015). Importantly, the meal was able to stabilize blood iCa concentrations during and after exercise. Indeed, iCa remained elevated above that of the CON diet for ~ 7 hours, spanning the period from the start of the first exercise session until 2 hours of recovery after EX2. The pre-exercise calcium-rich meal was equally effective in preventing the decline in iCa over exercise in each session, showing that the effect can be repeated.

The improved maintenance of iCa with the CAL trial was associated with lower PTH concentrations over the trial day, and an attenuation of the increase in PTH associated with exercise, particularly around the second session. The duration and periodicity of exposure to elevations in PTH govern the net effect on bone mass, with an intermittent increase in PTH stimulating bone formation whereas prolonged continuous exposure to high levels tips the balance in favor of resorption (Silva et al. 2015). In clinical situations, PTH concentrations should be assessed in conjunction with Vitamin D status and calcium intake, as Vitamin D deficiency has a secondary effect on PTH (Bonjour et al. 2014). We note that Vitamin D status was insufficient in 30% of our sample, likely due to the timing of our study coinciding with the annual seasonal nadir. Nevertheless, this is unlikely to have affected our findings given the crossover design of the study, and the absence of frank deficiency within our group.

Although further investigation is needed, it is likely that the PTH response to the CAL supplementation in the current study is indicative of less bone resorption (Townsend et al. 2016). Indeed, the increase in β-CTX-I seen with CON was cumulative and relatively prolonged, spanning the first exercise bout until 2 hours after the final exercise session was completed; a period of 6-7 hours. In contrast, β-CTX-I was unchanged from baseline for CAL and was higher at only two time points, a period spanning around 2 hours. Our findings of an attenuation of the β-CTX-I response to exercise following calcium supplementation is in agreement with the results of several studies of single exercise bouts in which the timing of calcium intake has been designed to allow gut release during the early exercise period (Guillemant et al. 2004, Haakonssen et al. 2015, Kohrt et al. 2018, Wherry et al. 2021a).

Our study included blood measurements of osteocalcin, a protein thought to be primarily synthesized by osteoblasts and often used as a marker for bone turnover (Ivaska et al. 2004). Indeed, we investigated both total osteocalcin concentrations (tOC), which includes the Vitamin K-stimulated carboxylated form (cOC) with high bone affinity, and its under-carboxylated form (ucOC) which is receiving attention for a range of endocrine effects (Wang et al. 2021). In the current study, we observed small but significant increases in tOC and ucOC associated with each exercise bout, and a

minor increase in the ratio of ucOC:tOC after the second bout, but no differential effect of the pre-exercise calcium intake on these changes. The acute post-exercise response is consistent with previous research (Parker et al. 2019, Hiam et al. 2021), with the lack of difference between CAL and CON supporting the observation that OC is likely most influenced by perturbations to energy and carbohydrate availability during exercise (Heikura et al. 2019, Fensham et al. 2021a), which were matched in the present study, rather than acute calcium concentrations. OC has been noted to increase in response to prolonged rowing training without feeding in both male (Jurimae et al. 2011) and female (Jurimae et al. 2011, Jurimae et al. 2016).

The limitations of this study, including the focus on elite male athletes, (necessitated by Covid-19 restrictions on the interstate travel of their female counterparts), as well as the small but clinically insignificant differences in the macronutrient and energy intake of the trial day diets are noted. The reliance on systemic markers of bone turnover which may provide an acute picture of change but not the long-range implications was also a limitation. We also note that P1NP is considered the preferred marker of bone formation (Dolan et al. 2020) but the time course of change for this marker (days vs hours) (Rantalainen et al. 2009) was incompatible with the current protocol. Indeed, in the previous study from our group involving highly trained cyclists (Haakonssen et al. 2015), pre-exercise Ca intake did not have an effect on the PINP response to exercise, despite a clear attenuation of the increase in β-CTX-1. Nevertheless, we identify many strengths including the involvement of elite athletes, the real-world application of the study theme to many highly trained athletes, and the involvement of holistic dietary strategies to address impairments of bone remodeling that are of relevance to health and performance.

9.2 Reflections

The research undertaken in the preparation of this thesis has been highly satisfying, given that the research questions arose directly from clinical challenges experienced in my role as Lead Sports Nutrition service provider to an elite rowing program. The practical applications derived from data collection have created personal and professional value. Completing a PhD while undertaking a full-time servicing role in elite sport was, perhaps, foolhardy and created immense challenges. Nevertheless, it was made worthwhile by the willing involvement of athletes, coaches and support team in many activities and their appreciation of the outcomes. The research questions arising from elite athletes, although often niche, are highly impactful in the sub-world of the sport. Finding a way to integrate research within the sport service model is critical to the systematic improvement of practice.

My first week with the elite rowing program involved a debrief of the previous season in which it was stated that nutrition practices were optimised and would need additional attention in the next cycle. Rather, the focus of the next campaign would be to reduce the loss of training time to injury,

primarily lower back and rib stress injuries. Immediately, I saw this as an opportunity to work in a different way, to hear the key concerns of rowing and examine how my professional domain could help with their identified problem.

The initial research brief for my doctoral work involved developing an understanding of BMD and its relationship to nutrition factors in rowers. I uncovered two key issues: relatively high rates of LEA among the rowing cohort, and its presence within the group that I had least suspected to be at risk. Typically, most attention around LEA is directed to weight-sensitive and weight-division sports, such as lightweight rowing. However, I discovered that heavyweight male rowers, despite consuming massive food volumes were the cohort most often failing to meet the energy cost of their training. Several practical actions emanated from this finding. The first involved advocacy to provide the budget and facilities to enable greater access to nutrition support within the daily training environment. Embedding the dietitian in domestic and international travel included the planning, expense and execution of additional food provision within competition and accommodation venues, often achieved via creative and makeshift use of tents, tables, fridges and generators. Although the nutrition service initially aimed to target individuals with suspected or identified LEA, it became clear that systemic changes were needed to support the whole team. An observation was made that although the training volume was higher than in previous cycles, better access to food seemed to allow the athletes to better absorb the increase workload. Protocols to refine and systematise the measurement of RMR within the elite sports environment were developed largely to assess the LEA challenge within rowing. This became an exemplar for other organisations within the high-performance sports system in Australia, leading to its uptake within other sports as well as the initiation of other doctoral programs to enhance the conduct and interpretation of this measurement.

The disadvantage of creating a successful activity is the ongoing requirement to meet the time and resource demands of its popular use. The validation of the screening questionnaire for LEA in males was primarily motivated by the desire to reduce the burden associated with undertaking RMR measurements (a lengthy early morning activity necessitating a rest day or missed training session) by targeting its use to athletes identified as high risk of problematic LEA exposure. The opportunity to collaborate with other centres to develop and validate the screening tool, including international colleagues who were involved in developing the LEAF-Q for female athletes, was timely and welcome. Although it was rational to include questions about sex drive in the first iteration of question bank, we were advised by external experts that they would cause discomfort and potentially produce flawed data. Although we later reversed this decision and recognised the importance of this information in identifying the risk of LEA, it delayed the progress of the questionnaire by several years! We also recognised the challenge of conducting a multicentre study

involving different countries, languages, equipment and methodologies. This was the most challenging study by far.

The second finding of note – and concern – from the systematic assessment of rower health supported by my doctoral program was the trend for a reduction in BMD over the course of an Olympic cycle. This surveillance uncovered case studies in which athletes who started the cycle with normal BMD suffered a stepwise decrease in BMD over the course of their career, with some reaching scores considered osteopenic. Our nutrition support program targeting LEA, which we had considered the primary cause, appeared to provide benefits for the BMD of female rowers. However, in the case of male rowers, resolution of the reductions in spine BMD did not transfer to the proximal femur BMD, leaving us to search for other strategies, unrelated to energy or CHO availability, to arrest its ongoing decline. This was one of the key reasons for undertaking Study 3, acute calcium intake prior to training, based on findings that had been achieved by my old sports nutrition team at the Australian Institute of Sport with cyclists. Although the results of this work involve an acute observation of bone markers, the strategy of integrating calcium-rich foods into a pre-training meal is practical and supportive of other sports nutrition goals. Therefore, chronic application doesn't not pose any disadvantages. The service team will also add bone loading strategies to support BMD.

9.3 Future Directions

Further research is required to confirm if rib BMD is useful as a marker of RSI risk in rowing populations and to develop normative data according to sex and weight category. Understanding the time course of change with interventions (dietary or bone loading) and whether increases in rib BMD influence injury risk is also important. Sex differences were seen in the association of bone parameters with RSI history in the current study; lightweight men with injury history showed lower bone mass, femur and rib BMD, with the latter also seen in heavyweight women with injury risk. Meanwhile, heavyweight men and lightweight women with injury risk did not share these characteristics. Whether these differences are specific to the population studied or whether they are generalisable to the broader rowing communities could be further investigated. Similarly, the impact of graded diet restriction on rib BMD was different between sexes with males showing an inverse relationship between diet restriction and rib BMD and females having a U-shaped curve with the lowest rib BMD at a moderate level of diet restriction. The reasons for this are unclear. It is possible that using more precise tools to identify LEA such as resting metabolic rate would provide a clearer answer to this question. Whether the normal status for vitamin D and K and high dietary calcium intake is specific to the Australian rowing population included in this study or whether it is a broader

attribute of rowing populations needs to be confirmed, as does the role of these nutrients, whilst not showing a relationship with RSI in the current research, in other cohorts. Future prospective research should aim to determine associations with risk of multiple compared with single RSI. The influence of rowing specific training on BMD, independent of nutrition factors, needs further clarification.

Further research is required to understand the interaction between the immune system and EA in athlete populations. A recent review of the complex relationship between nutrition and immune tolerance/resistance has recently proposed that energy restriction per se may not increase illness risk, and that previous associations reported in studies of athletic populations may be mediated by a common co-morbidity such as higher ratings of psychological stress (Walsh 2019). Indeed, one study has reported an apparent disconnect between EA and the occurrence of upper respiratory infections in athletes who commenced high-intensity interval training (Hanstock et al. 2019). This is of practical importance to Australian rowers who prepare for northern hemisphere competition during the winter cold/flu season.

Whilst the validation process for the LEAM-Q was prolonged and extensive, further refinement and investigation would be beneficial. Questions regarding weight flux, an increased detail into training load assessment, questions regarding self-reported energy restriction and simultaneous collection of disordered eating and exercise dependence screening would help to further delineate LEA, EHMC and DE. It is possible that the inclusion of injury questions that are specific to sport of the questionnaire participant would also be beneficial. Given that sex drive questions were added in phase two of data collection, including these in a larger sample would be of benefit. Sex drive and its potential relationship to LEA in both males and females is worthy of further investigation; indeed, it may be useful to interrogate sex drive in female populations as an adjunct to information on menstrual function or to address situations where the use of hormonal contraceptives interferes with an assessment of menstrual status. Indeed, females with anorexia nervosa are reported to experience lower sex drive (Piontek et al. 2019).

While the best possible effort was made to characterise clinical markers identifying LEA in the present study, further research is required to better identify thresholds indicative of perturbation in male athletes. In this study, the lowest or highest quartile was used for several variables where sub-clinical deficiency is likely to be important, but reference ranges for the marker are not yet available. Consistency in these cut-points will be important for future research and it is encouraging to see the development of this characteristic for testosterone (Fredericson et al. 2021). Ratios of cortisol:insulin and free testosterone:cortisol were significantly different between LEA cases and controls in our study, however, inconsistency of measurement units in previous research makes comparisons or the development of normative ranges challenging. While the overall data set was relatively large, key

variables such as insulin, testosterone, and cortisol were only included in version 2 of the study and as such the sample size is much smaller for these key areas.

Previous research has shown that male athletes with higher exercise energy expenditure have lower EA (Koehler et al. 2013) and males with eating disorders are more likely to have a focus on exercise rather than diet as a weight loss strategy (Silla et al. 2021). Questions around training load and intensity have been successful in identifying male athletes with low testosterone (Hackney et al. 2017) and exercise dependence with low testosterone cortisol ratio and high cortisol insulin ratio (Torstveit et al. 2019). Further work from should focus on a new version of the LEAM-Q that extends the investigation of sex drive, with the addition of information around flux of body mass/composition and training load. The current LEAM-Q included a question on training hours which was associated with aspects of sex drive. Given the diversity of the sports included in this investigation, this question was inadequate to capture the range of training loads that might be consider low to extreme in a specific sport. The further development of questions of this nature may be worthwhile and have been included in the amended version of the LEAM-Q questionnaire.

Further research into pre-exercise calcium intake could clarify the minimum effective dose, since dietary intake of 1,000 mg calcium prior to several training sessions per day may be difficult to achieve for rowers who are vegan or have lower energy requirements than heavyweight males. The potential issues around continued use of calcium supplements should be considered. Whether this dose is influenced by body mass, fat free mass, sex or exercise intensity or duration also need to be clarified. Future research could monitor changes to bone formation markers over the days post exercise and better identify the longer-term changes in β-CTX-I. Prospective studies of long-term pre-exercise calcium support are not without challenges but would be welcome.

9.4 Conclusions

Bone stress injuries are multi-factorial, but several nutrition factors may be important contributors to risk. In elite rowing populations, monitoring BMD at the AP spine, total femur or rib sites may provide insight into relative risk of individuals. Diet restriction appears related to BMD in rowers and care should be taken to ensure that energy intake is sufficient to meet the heavy training demands. Monitoring the menstrual status of female rowers may also be important in identifying LEA, while in males, sex drive is likely to be the best indicator of LEA outside of blood biomarkers.

The current study of elite Australian rowers across sex and weight class categories found that BMD, calcium intake and vitamin D status typically met population guidelines and standards associated with good health. Nutritional strategies to support injury prevention should focus on energy

availability and its contribution to health and function, including menstrual status. Monitoring of BMD, including rib measures, over time or against future sports-specific targets may be helpful in allowing the early detection of the risk of RSI or as a measure of the success of strategies to prevent/manage such injuries.

Pre-exercise intake of calcium-rich foods can lower markers of bone resorption during and following exercise. Specifically, the repeated intake of calcium-rich meals prior to training sessions undertaken within the same day has a cumulative and sustained effect on the stabilization of blood iCa during exercise. In turn, this reduces the PTH response to exercise, likely leading to attenuating the increase in markers of bone resorption. Pre-exercise calcium intake is a simple strategy that can largely be achieved through diet manipulation and may provide an additional strategy to be used alongside EA adequacy to reduce risk of adverse bone health changes and injury.

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11. Research Portfolio Appendix

11.1 Statement of Contribution of Others

Study 1.

Lundy B, Suni V, Drew M, Trease L, Burke LM. Nutrition factors associated with rib stress injury history in elite rowers. Journal of Science and Medicine in Sport. 2022. submitted

Contribution statement: BL was primarily responsible for the research question, design, data collection, assembly of data, interpretation of the analysis, drafting, revising and the final manuscript. VS was primarily responsible for the data analysis with contribution from MD. VS contributed to the interpretation of the analysis, manuscript drafts including figures. MD contributed to the research question and design, data analysis and manuscript review. LT contributed to the data collection, interpretation and manuscript review. LB was involved in all aspects of the study and contributed to the manuscript preparation, review and final approval.

Approximate percentage contributions: B Lundy: 70% V Suni: 15% M Drew: 5% L Trease 5% L Burke: 5%

I acknowledge that my contribution to the above paper is 70%

Bronwen Lundy, 30th March 2022

As principal supervisor of this project I certify the above contributions are true and correct

LM Burke,

Co-Authors

Veronika Suni

Michael Drew

Larissa Trease

Study 2.

Lundy B, Torstveit MK, Stenqvist TB, Burke LM, Garthe I, Slater G, Ritz, C., Melin, A. K. Screening for low energy availability in male athletes: attempted validation of LEAM-Q. Nutrients. 2022; submitted

Contribution statement: BL, LB, AKM and MT were primarily responsible for the research question and study design, data collection and collation was shared by all authors for their research location. BL was primarily responsible for the collation of data form each centre and data collection and collation for the Australian cohort, the interpretation of the analysis, drafting, revising and the final manuscript. TS contributed to the data analysis and interpretation and review of the manuscript. CR was primarily responsible for the statistical analysis and contributed to manuscript drafts. IG contributed to data collection and the direction of the analysis. GS contributed to data collection and manuscript review. LB was involved in all aspects of the study and contributed to the manuscript preparation, review and final approval.

Approximate percentage contributions: B Lundy: 60%, M Torstveit 5%, Stenqvist 5%, L Burke: 10%, 10% A Melin, 5% C Ritz 2.5% G Slater 2.5 % I Garthe

I acknowledge that my contribution to the above paper is 60%

Bronwen Lundy, 30th March 2022

As principal supervisor of this project I certify the above contributions are true and correct

LM Burke,

Co-Authors

Monika Torstveit 

Thomas Stenqvist 

Ina Garthe [redacted]

Gary Slater [redacted]

Christian Ritz [redacted]

Anna Melin [redacted]

Study 3.

Lundy B, McKay AKA, Fensham NC, Tee N, Anderson B, Morabito A, Sim M, Ross, M, Ackerman K, Burke, L. The impact of acute calcium intake on bone turnover markers during a training day in elite male rowers. Med Sci Sports Exerc. 2022; submitted

Contribution statement: BL was primarily responsible for recruitment, diet design and standardisation, data collation, interpretation and presentation, drafting, revising and the final manuscript. AMc was primarily responsible for the data analysis with contribution from NF. NF additionally contributed to the manuscript draft and review. AMc, NT and MS were responsible for blood analysis and contributed to the manuscript draft and reviews, BA contributed to the diet standardisation, data collection and manuscript draft and review and AM contributed data collection and manuscript review. MD contributed to the research question and design, data analysis and manuscript review. MR was primarily responsible to study planning and data collects and contributed to manuscript review. KA provided expert opinion and manuscript review. LB was involved in all aspects of the study and contributed to the manuscript preparation, review and final approval.

Approximate percentage contributions: B Lundy: 60%, A McKay 10%, N Fensham 2.5% N Tee 5%, B Anderson, 2.5%, A Morabito, 2.5%, M Sim, 2.5%, M Ross 2.5%, K Ackerman 2.5%, L Burke: 10%

I acknowledge that my contribution to the above paper is 60%

Bronwen Lundy, 30th March 2022

As principal supervisor of this project I certify the above contributions are true and correct

LM Burke

Co-Authors

A McKay [redacted]

N Fensham [redacted]

N Tee [redacted]

B Anderson [redacted]

A Morabito [redacted]

M Sim [redacted]

M Ross [redacted]

K Ackerman

11.2 Ethics Approvals

Australian Institute of Sport

MINUTE

TO: Ms Bronwyn Lundy CC:

FROM: Ms Helene Rushby

SUBJECT: Approval from AIS Ethics Committee DATE: 18th February 2013

On the 12th of February 2013, the AIS Ethics Committee gave consideration to your submission titled "Identifying risk factors for rib stress fractures in Elite Australian Rowers". The Committee saw no ethical reason why your project should not proceed subject to:

- The inclusion of the time requirement for participants and a description of the stress tests in the information to participants
- The inclusion of the paragraph "may elect in writing to have results sent to rowing" in the information to participants.
- The inclusion of information surrounding blood sampling in the information to participants

The approval number for this project: 20130208

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

Any proposed changes to the research design,
Any adverse events that may occur,

Researchers are required to submit annual status reports and final reports to the secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guidelines" for ethics submissions.

If you have any questions regarding this matter, please don't hesitate to contact me on (02) 6214 1577

[redacted]

Sincerely
Helene Rushby

Australian Institute of Sport

MINUTE

TO: Bronwen Lundy CC:

FROM: Ms Joanne Allen

SUBJECT: Approval from AIS Ethics Committee DATE: 11 December 2013

On the 10th of December 2013, the AIS Ethics Committee gave consideration to your to vary your study titled *"Identifying risk factors for rib stress fractures in elite Australian rowers".* The Committee saw no ethical reason why your project should not proceed.

The approval number for this project: 20130208

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

Any proposed changes to the research design,
Any adverse events that may occur,

Researchers are required to submit **annual status reports** and **final reports** to the secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guidelines" for ethics submissions.

If you have any questions regarding this matter, please don't hesitate to contact me on (02) 6214 1577.

Sincerely
Joanne Allen
A/g Secretary, AIS EC (Acting)

AIS | Ethics

MINUTE: 3 DECEMBER 2021

TO: Bronwen Lundy

FROM: Michael Gillard, AIS Ethics Committee Secretary

SUBMISSION TITLE: Nutrition contributors to rib stress injury in elite rowers

The project extension request to your previously approved research submission (titled above) has been considered. The specified extension does not give rise to any ethical reason why the project should not recommence as proposed.

Please note your **new ethics approval number** and the postponed ethics approval expiry date, based on the newly anticipated project completion date outlined in your extension request:

Ethics approval number: **20130208R2**
Ethics approval expiry: **30 June 2022**

As the Principal Researcher, please advise all researchers involved in the study of the EC approval and any conditions of approval stated above. Please also advise the EC immediately (via the Secretary) of:

- Any proposed changes to the research design
- Any adverse events that occur whilst carrying out your research study
- Premature termination of the project
- Project completion.

Researchers are responsible for submitting the required annual status reports and project completion reports to the EC Secretary. Details of report requirements can be found online at:

https://www.ais.gov.au/research-submissions

After approval expires, you will need to submit an extension request to continue the study if required. If you have any questions regarding this matter, please contact me.

Sincerely,

[redacted]

Michael Gillard
AIS Ethics Committee Secretary
ethics@ausport.gov.au

Ethics

MINUTE: 28 JANUARY 2022

TO: Bronwen Lundy

FROM: Michael Gillard, AIS Ethics Committee Secretary

SUBMISSION TITLE: Identifying risk factors for rib stress fractures in Elite Australian Rowers

The **minor variation request** to your original research submission (titled above) has been considered. The risks associated with the specified changes to the research project do not give rise to any ethical reason why the project should not proceed as proposed in this minor variation submission.

Please note your **new ethics approval number**, and the ethics approval expiry date.

Ethics approval number: **20130208R3**
Ethics approval expiry: **30 June 2022**

As the Principal Researcher, please advise all researchers involved in the study of the EC approval and any conditions of approval stated above.

Please also advise the EC immediately (via the Secretary) of:

- Any proposed changes to the research design
- Any adverse events that occur whilst carrying out your research study
- Premature termination of the project
- Project completion.

Researchers are responsible for submitting the required annual status reports and project completion reports to the EC Secretary. Details of report requirements can be found online at:

https://www.ais.gov.au/research-submissions

After approval expires, you will need to submit an extension request to continue the study if required. If you have any questions regarding this matter, please contact me.

Sincerely,

Michael Gillard
AIS Ethics Committee Secretary
ethics@ausport.gov.au

Australian Institute of Sport

MINUTE

TO:	Ms Bronwen Lundy	CC:	
FROM:	Ms Helene Rushby		
SUBJECT:	Approval from AIS Ethics Committee	DATE:	3.11.16

On the 11th October 2016, the AIS Ethics Committee gave consideration to your submission titled ***"Validation of LEAM-Q in male athletes".*** The Committee saw no ethical reason why your project should not proceed.

The approval number for this project is: 20161006

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

Any proposed changes to the research design,
Any adverse events that may occur,

Researchers are required to submit **annual status reports** and **final reports** to the secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guidelines" for ethics submissions.

Please note the approval for this submission expires on the 30th December 2018 after which time an extension will need to be sought.

If you have any questions regarding this matter, please don't hesitate to contact me on (02) 6214 1577

Sincerely

[redacted]

Heléne Rushby
Secretary, AIS EC

Australian Institute of Sport

MINUTE

TO: Bronwen Lundy DATE: 9th September 2019

FROM: Myfanwy Galloway (AIS Ethics Committee Secretary)

SUBJECT: Minor Variation to – **"LEAM-Q Validation"** (20161006)

On the 9th September 2019, the AIS Ethics Committee Secretary gave consideration to your minor variation request to the submission **"LEAM-Q Validation"** The Committee Secretary saw no ethical reason why this variation should not be approved.

The approval number for this project is: 20161006

It is a requirement of the AIS Ethics Committee that the Principal Researcher (you) advise all researchers involved in the study of the Ethics Committee approval and any conditions of that approval. You are also required to advise the Ethics Committee immediately (via the Secretary) of:

- Any proposed changes to the research design;
- Any adverse events that might have occurred.

Researchers are required to submit annual status reports and final reports to the Secretary of the AIS Ethics Committee. Details of status report requirements are contained in the "Guideline" for ethics submissions.

Please note that approval for this submission expires on the 31st December 2020 after which time an extension will need to be sought.

If you have any questions regarding this matter, please contact me on (02) 6214 1791

Sincerely,

Myfanwy Galloway
Secretary, AIS Ethics Committee

AIS Ethics

MINUTE: 3 DECEMBER 2021

TO: Bronwen Lundy

FROM: Michael Gillard, AIS Ethics Committee Secretary

SUBMISSION TITLE: Validation of LEAM-Q in male athletes

The project extension request to your previously approved research submission (titled above) has been considered. The specified extension does not give rise to any ethical reason why the project should not recommence as proposed.

Please note your **new ethics approval number** and the postponed ethics approval expiry date, based on the newly anticipated project completion date outlined in your extension request:

Ethics approval number: **20161006R3**
Ethics approval expiry: **30 June 2022**

As the Principal Researcher, please advise all researchers involved in the study of the EC approval and any conditions of approval stated above. Please also advise the EC immediately (via the Secretary) of:

- Any proposed changes to the research design
- Any adverse events that occur whilst carrying out your research study
- Premature termination of the project
- Project completion.

Researchers are responsible for submitting the required annual status reports and project completion reports to the EC Secretary. Details of report requirements can be found online at:

https://www.ais.gov.au/research-submissions

After approval expires, you will need to submit an extension request to continue the study if required. If you have any questions regarding this matter, please contact me.

Sincerely,

[redacted]

Michael Gillard
AIS Ethics Committee Secretary
ethics@ausport.gov.au

Ethics

MINUTE: 14th October 2020

TO: Bronwen Lundy

FROM: Rikki Belder, AIS Ethics Committee Secretary

SUBMISSION TITLE: The impact of acute calcium intake on bone markers and iron status, during repeated training sessions and recovery in elite rowers.

The AIS Ethics Committee (EC) have considered your research submission, titled above. The EC does not see any ethical reason why the project should not proceed as specified in your submission.

Ethics approval number: **20200905**
Ethics approval expiry: **30th April 2022**

Please note that approval is subject to the following conditions:

- Condition 1: Please provide your clinical trial registration number when it is received.

As the Principal Researcher, please advise all researchers involved in the study of the EC approval and any conditions of approval stated above. Please also advise the EC immediately (via the Secretary) of:

- Any proposed changes to the research design
- Any adverse events that occur whilst carrying out your research study
- Premature termination of the project
- Project completion.

Researchers are responsible for submitting the required annual status reports and project completion reports to the EC Secretary. Details of report requirements can be found online at:

https://www.ais.gov.au/research-submissions

After approval expires, you will need to submit an extension request to continue the study if required. If you have any questions regarding this matter, please contact me.

Sincerely,

[redacted]

Rikki Belder
AIS Ethics Committee Secretary
ethics@ausport.gov.au