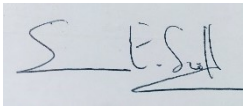
An Investigation Into The Effects Of Brief Diverse Exercise On Bone

Submitted by Suzanne Elaine Scott, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Sport and Health Sciences, December 2020.

I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

(Signature) 

……

Acknowledgements

Firstly, I would like to thank Professor Peter Krustrup, who took me on as a PhD student, and has continued to include me in his research circle, funding the first three years of this part-time thesis from a FIFA grant, and subsequently funding biomarker assays from a grant from the University of Odense. Thank you for your inspiration and for setting me on the path of exercise research.

Thank you to Associate Professor Karen Knapp, and Professor Joanna Bowtell, from the University of Exeter (UoE), who stoically assumed the role of Supervisors, turned up to football pitches and dance studios to support data gathering, gave up their time whenever it was required, offered advice and provided brilliant academic stewardship, without ever exhibiting fatigue (central, peripheral or as yet uncharacterised). You guided me towards bone as a theme, kept me (and the length of my sentences) on track, and have advised and supported me over many more years than I am sure either of you ever thought you would have to. Thank you, Karen, for never being fazed, whatever was suggested, and Jo, for presenting at the ECS, when I was unexpectedly still at a football tournament in Russia. You both are, and have been, extraordinary.

It would never have been possible to complete studies in this thesis without the assistance of other researchers from UoE: Dr Jon Fulford, who helped make sense of acceleration, MRI (and other) data, and with Associate Professor Karen Knapp, helped secure Bridge The Gaps funding which enabled the dance grid feasibility study. Thanks are also due to Dr Sarah Jackman and Dr Rosey Davies, UoE, who mentored me through the early days of research, and Dr Luke Connolly, now University of Plymouth, who ran football, grid training, and data collection alongside me, and David Childs and Jamie Blackwell, from the School of Sport and Health Sciences at UoE, who de-mystified dry ice, research consumables and laboratory procedures. A special thank you to Dr Karja Seidel, University of Westminster, who provided access to the university's laboratories to process samples during field work.

Thank you to Anna Brodrick-Turgoose, at the Central School of Ballet (CSB), for her peerless collaboration and dance research expertise, and for letting us install a pop-up venipuncture lab in her treatment room, take over ballet studios for mass data gathering and for never being less than 100% enthusiastic. To you, to Sara Matthews, who was then Principal of CSB, the

staff, and the dancers of CSB, I am deeply indebted. Thank you for the trust you placed in me and your commitment to the training study.

Others have provided help with equipment: the team at Catapult, who lent me motion sensor units, and Mike Lee, who came along with them, supported the first training session, and helped us get dancers into vests made for rugby players. Thanks are also due to GPSport, who provided units for the feasibility study, and I would like to thank Dr Jon Tang and Profesor William Fraser, who undertook bone biomarker assays at the University of East Anglia, when I was self-funding and struggling to achieve this. Most recently, I have received invaluable help in formatting this document from Dr Jo Varley-Campbell, University College London, who is as magical at Word as a certain boy wizard is at quidditch.

None of the studies would have been possible without the participants, who turned up, provided data, trained, and then provided more data. Thank you, for what you so generously gave of yourselves, and your time.

Finally, I would like to thank my family, Lotte and Gus Scott, and friends Sallie Lennon Eaton and Margaret Duston, who stood by with clipboards and kept the improvised dance laboratory at CSB running, and above all, thank you to my husband, Jock Scott, who turned citizen researcher and has not stopped, or stopped supporting me, ever.

And the very last word is for my father, William Emrys Watson (July 1925 – March 2020), to whom this thesis is dedicated. Thank you for everything.

Abstract

Rationale

Physical inactivity (PA) is a modifiable risk factor for reduced bone mineral density (BMD) associated with sedentary behaviour, and bone impairment in states of low energy availability has been demonstrated in lean athletes, such as ballet dancers. Team sport is shown to provide an anabolic stimulus to bone; however, it is not known whether there is an osteogenic effect at reduced exercise duration. To address this, we investigated the effects of brief bouts of team sports activity in two populations associated with potential impairment to bone health: sedentary females and ballet dancers, to evaluate the potential for an osteogenic effect of low-volume, diverse exercise.

Methods

Brief bouts of small-sided football (SSG) were investigated in sedentary females (SF) and a novel diverse HIIT protocol (DM-HIIT) in SF and female (DF) and male ballet dancers (MBD).

Chronic effects of exercise were measured with DXA (SSG) and calcaneal QUS (DM-HIIT) and bone turnover markers (BTM) were used to characterise acute responses to exercise (SSG) and background bone metabolism (SSG, DM-HIIT). Gonadal hormones were measured at rest (DF, MBD). Locomotor profile was characterised by distance using GPS activity domains (SSG) and by duration for tri-orthogonal acceleration data (DM-HIIT) in bands from low (1 – 1.5 g) to high (> 3 g) threshold.

Results

Whereas diverse exercise as SSG increased total hip BMD in SF and acutely elevated bone formation, there was no effect of 12 weeks DM-HIIT on calcaneal bone (SF, DF, MBD) or background BTMs (SF, MBD), however in DF bone resorption decreased and accelerations > 3 g significantly increased after 12 weeks training. Gonadal hormone profile (DF) indicated menstrual dysfunction in female dance cohorts. Participants achieved HIIT intensity thresholds during DM-HIIT and there was a direction-specific effect on vertical

accelerations, which increased after 12 weeks training in all bands (SF, DF, MBD), and significantly above 3 g (highest threshold) in female dancers.

Conclusion

We demonstrated an osteogenic effect on hip BMD and elevation in bone formation for low-duration SSG, and background bone resorption was reduced after 12 weeks diverse HIIT in female ballet dancers. However, bone formation did not increase, which was speculatively attributed to impairment in gonadotropic hormone profile in the dancers. High-intensity accelerations are proposed to represent the potential osteogenic signal elicited by diverse HIIT. Longer (> 4 months) studies using pQCT/ DXA and BTMs to characterise acute, as well as background, bone metabolic responses are recommended to investigate the potential of brief, diverse HIIT in populations who may benefit from exercise to improve bone health.

Table of contents

List of Tables

List of Figures

Author's declaration

The author's contribution to research described this document is as follows:

Study 1 (Chapter 4) : 'Effects of 16 weeks Small-Sided Football or Whole-Body Vibration on Bone - A Randomised Controlled Intervention with Brief Diverse Exercise in Sedentary Premenopausal Females'

Data collection: May 2012 – September 2013

The study was initiated by the author's Supervisors, Professor Joanna Bowtell and Associate Professor Karen Knapp, and former Supervisor Professor Peter Krustrup. The author took no part in study design, grant application or ethics. The author assisted with recruitment and gathering of primary data, as detailed, including exceptions, in Appendix 4, conducted training sessions in the football arm of the intervention, in collaboration with other researchers identified in Appendix 4, undertook extraction of data from data bases and software pertaining to analysis presented in this document, and performed all analyses described (Chapter 4, Results, 4.3). Co-authorship of publications identified in Appendix 5, related to this study, involved contribution to data analysis and preparation, and writing journal submissions in collaboration with the research team.

Study 2 (Chapter 5) : 'Development of Brief Diverse Movement High-Intensity Interval Training (DM-HIIT): The Grid Feasibility Study'

Data collection: May 2013 – October 2013

The author wrote the funding application and after this was secured, the ethics application, and all documents pertaining to recruitment and data gathering, excepting validated and pre-existing assessment tools. The protocol was designed, including the audiovisual presentation, by the author and all training sessions conducted under the author's supervision. The author performed primary data gathering as described (Chapter 5, Methods 5.2), assisted during venipuncture by members of the research team identified in Appendix 4, and undertook all subsequent analyses and writing up of data. The

author wrote the abstracts and presentations relating to this research, with feedback and contributions from the research team, and carried out subsequent presentations, unless otherwise identified (see Appendix 5).

Study 3 (Chapter 6) : ‘Characterising Locomotor Profile and Acute Physiological Demands of Diverse Movement-HIIT (DM-HIIT) In Sedentary Women And Female Dancers’

Data collection: September 2013 (Female Dancers); March 2017 -April 2017 (Sedentary Females)

The author wrote the ethics application, designed the protocol and audiovisual presentation of training, conducted exercise sessions and gathered primary data, as detailed in Appendix 4; performed data extraction and analysis, except for acceleration data, which were initially examined by co-researcher, Dr Jon Fulford, using Microsoft Excel scripts. Subsequent analysis was completed by the author, with support from this co-researcher.

Study 4 (Chapter 7) ‘Effects of 12 Weeks Diverse HIIT On Bone: A Randomised Controlled Home Exercise Intervention In Sedentary Premenopausal Females’

Data collection: February 2015 – December 2015

The author collaborated with Dr Luke Connolly on the design of the study and ethics application, and initially shared recruitment procedures, before in principal assuming this role and primary data collection; uploading to the data base was undertaken by Dr Connolly. The author conducted all exercise training, and the diverse HIIT protocol described, and its audiovisual presentation, were originated by the author. Data collection as described in Chapter 7, Methods 7.2, was undertaken by the author and Dr Connolly, assisted by others as detailed in Appendix 4, and the author initiated bone biomarker assay and funded the costs of this. Professor Peter Krustrup subsequently provided reimbursement of costs through a grant from the University Of Odense. Analyses for results described in Chapter 7, Results 4.3 were performed by the author, except for assay procedures (see Appendix 4) and threshold banding of acceleration data, as described for Chapter 6 above. The publication from this study (Appendix 5) was initiated, and in large part

authored, by Dr Luke Connolly; the present author contributed the rationale for the exercise protocol, and relevant data pertaining to participant training indices.

Study 5 (Chapter 8) : ‘Effects Of 12 Weeks Supervised Diverse HIIT On Bone Metabolism And Calcaneal QUS In Young Male and Female Ballet Dancers’

Data collection: September 2014 – January 2015

The author wrote the ethics application and designed the protocol and audiovisual presentation of training material. The author performed all primary data gathering, assisted by the co-researchers and Supervisors identified in Appendix 4, and was assisted in venipuncture by Professor Joanna Bowtell and Associate Professor Karen Knapp. All training sessions were conducted by the author at CSB, assisted by staff at the school, principally the Clinical Lead, physiotherapist Anna Brodrick Turgoose, and dance analyst Stephanie D’Ath. Data extraction and analysis to provide results (Chapter 8, Results 8.3), were undertaken by the author, except for initial analysis of acceleration data, by Dr Jon Fulford. Presentations and abstracts pertaining to these, as detailed in Appendix 5, were written by the author, with collaborative support from Supervisors.

Definitions and abbreviations

AN	Anorexia nervosa	OP	Osteoporosis
AU	Arbitrary unit	PA	Physical activity
BLa	Blood lactate	P1NP	N-terminal propeptide of type-1 procollagen
BM	Body mass	QA	Quality assurance
BMI	Body mass index	QUS	Quantitative ultrasound
BP	Blood pressure	REDS	Relative Energy Deficiency-Sport
BMC	Bone mineral content	RPE	Rating of perceived effort
BMD	Bone mineral density	SAT	Subcutaneous adipose tissue
BTM	Bone turnover marker	SB	Sedentary behaviour
BUA	Broadband ultrasound attenuation	SHBG	Sex hormone binding globulin
COD	Change of direction	SOS	Speed of sound
CTX-1	C-terminal telopeptide of type 1 collagen	SSFT	Small-sided football training
CoV	Coefficient of variation	SSG	Small-sided games
CV	Cardiovascular	TAU	Training as usual
DM-HIIT	Diverse-Movement HIIT	T	Testosterone
DXA	Dual x-ray absorptiometry	VAS	Visual analogue scale
EA	Energy availability	VAT	Visceral adipose tissue
EB	Eating behaviour	WBV	Whole-body vibration
E2	Estradiol	WC	Waist circumference
FM	Fat mass	W:H	Waist to hip ratio
FSH	Follicle stimulating hormone	W:R	Work to rest ratio
GPS	Global positioning system	25(OH)D	25-hydroxycholecalciferol
HC	Hip circumference		

HIA	High-intensity activity
HIIT	High-intensity interval training
HPG	Hypothalamic-pituitary-gonadal axis
HR	Heart rate
LH	Luteinising hormone
LM	Lean mass
MAP	Mean arterial pressure
MEMS	Microelectricomechanical systems
MD	Menstrual dysfunction
MRI	Magnetic resonance imaging
MSK	Musculoskeletal
OC	Osteocalcin

1 Introduction

Physical activity is recognised as a non-pharmacological means of addressing noncommunicable diseases (NCDs), which were estimated in 2015 to have caused 71% of deaths worldwide (World Health Organisation, [WHO], 2018)[1]. Alongside a role for regular exercise in reducing incidence and severity of an array of so-called 'lifestyle' NCDs, there is increasing appreciation of the functional interdependence between muscle and bone, which exercise uniquely exploits.

However, characterising the dose-response to exercise presents fundamental and methodological challenges. Exercise is complex, i.e. rich in dimensionality, and therefore difficult to titrate and administer by dose, according to the pharmacological paradigm. Evidence from interventional studies may also be hard to assess using standard medical conventions, since neither researchers nor participants can easily be blinded as to who is being given exercise and who is not. This has led to critical debate about the quality of evidence provided by interventional exercise research (Kujala, 2009).

If 'exercise as medicine' is the answer, characterising responses to exercise in studies studies that are robust and comprehensive in design, is critical for developing a reliable and comprehensive evidence-base and to inform accurate prescription.

Therefore, taking bone as its theme, the global aim of research undertaken for this thesis is to investigate the effect of prescribing brief exercise to promote beneficial adaptation in bone. Specifically, the outcomes of applying diverse skeletal loading and intermittent high-intensity, in a novel exercise format, are described in different populations, to extend the existing evidence base for the role of exercise in improving bone health.

Contribution to knowledge of exercise effects on bone

1.1 Study 1

'Effects of 16 weeks Small-Sided Football or Whole-Body Vibration on Bone - A Randomised Controlled Intervention with Brief Diverse Exercise in Sedentary Premenopausal Females'

1.1.1 Background and rationale

Recreational football has been shown to improve bone health in premenopausal females (Krustrup, Helge, et al., 2018). Increased bone formation characterised by biomarkers of bone formation has been observed in elite football athletes (Jackman et al., 2013) and in sedentary females (Mohr et al., 2015). Previous studies have typically implemented high-volume training (~60-90 minutes, 3 x per week) over several months (Krustrup, Hansen, et al., 2010), to approximate training conditions associated with osteogenic effects. These include higher BMD in young and adolescent female players compared with age-matched non-footballers (Plaza-Carmona et al., 2016), improved bone geometry in adolescent female footballers (Lozano-Berges et al., 2018) and superior BMD and BMC at collegiate level, compared with non-football female athletes (Bellver, Del Rio, Jovell, Drobnic, & Trilla, 2019). However, it is not known if there is an osteogenic effect of training at a lower volume.

In contrast with football, whole-body vibration (WBV) is a passive activity, which applies platform oscillations to elicit accelerations and ensuing reflex muscle contractions. Although WBV has been investigated in older populations to address osteoporosis and demonstrated improvement in hip BMD (Verschueren et al., 2004), evidence of an osteogenic effect in younger populations is controversial (Slatkovska, Alibhai, Beyene, & Cheung, 2010).

1.1.2 Investigative aim

We investigated whether there is an osteogenic effect of 16 weeks of low-volume exercise in sedentary females exposed to either recreational football played as small-sided games (SSG), or whole-body vibration, compared with controls.

1.1.3 Methods and approach

Sedentary females (25 – 45 y) were randomised to either control (*n* = 14), SSG (*n* = 13) or WBV (*n* = 16) and exercised for 13.5 min twice weekly (SSG; WBV). DXA was used to measure bone, and biomarkers of bone turnover were sampled at rest and after exercise, to characterise background and acute responses to training. Locomotor profile and heart rate during exercise were quantifed with GPS (SSG), to describe acceleration characteristics and distance covered in GPS activity categories.

1.1.4 Outcomes and impact

An osteogenic effect was observed for SSG, which increased total hip BMD in females, with no change in WBV and controls. This finding was accompanied by acute and delayed elevations in bone formation after SSG at baseline and after 16 weeks, with some evidence that ramping of WBV caused an increase in bone formation after 16 weeks, however no effect on BMD for WBV was found. A total of 43 women completed the study; attrition was high in all intervention groups (CON: 42%; SSG: 32%; WBV: 20%) and participant feedback indicated lack of time to attend (all arms of study), and not finding SSG enjoyable, as reasons for low-attendance and drop out.

Two papers have been published describing findings from this study: (Bowtell et al., 2016) (journal impact factor: 2.276; citescore: 3.600; 18 citations [20.12.2020]) and (Connolly et al., 2014) (journal impact factor: 5.2; citescore 6.3; 30 citations [20.12.2020]), to which the author contributed (Appendix 5.7), in addition to poster presentations (Appendix 5.2).

After demonstrating an osteogenic effect for low-volume team sports, but also that sedentary females perceived lack of time as a barrier to exercise, it was proposed to develop a diverse exercise protocol using team sports movements, and assess feasibility of brief bouts of training in sedentary females.

1.2 Study 2

'Development of Brief Diverse Movement High-Intensity Interval Training (DM-HIIT): The Grid Feasibility Study'

1.2.1 Background and rationale

High-intensity interval training (HIIT) has been demonstrated to benefit cardiometabolic health outcomes (Gibala, 2018) and is evidenced to improve indicators of cardiorespiratory fitness in patient populations (Weston, Wisløff, & Coombes, 2014). The HIIT approach to exercise has not been explicitly investigated to target bone outcomes, which may reflect a predominance for cycling and running in the activities selected in interventions. However, multidirectional and varying movement may be more osteogenic to bone, and this is supported by cross-sectional evidence in athletes of higher regional and whole-body BMD for long-term participation, in sports featuring high-impact and diverse movements (Carbuhn, Fernandez, Bragg, Green, & Crouse, 2010).

After demonstrating an osteogenic effect of SSG in sedentary premenopausal females (Study 1), a protocol was designed that harvested actions from team sports, such as intermittent shuttles runs, jumping and lateral cutting. These actions have been proposed to provide the stimulus to osteogenic effects observed in females in a recreational setting (Krustrup, Helge, et al., 2018) and are characteristic of locomotor profile in non-elite participation (Randers et al., 2010). Actions were combined with movements from dance, such as slow balances and controlled upper and lower limb gestures, to provide recovery and enhance intermittence, which are salient features in team sports activity profile (Castagna, D'Ottavio, & Abt, 2003). Exercise intensity was imposed according to an approach previously examined in sub-elite runners (Gunnarsson & Bangsbo, 2012), which reported significant cardiometabolic adaptation to 10 – 20 – 30 s of high-, medium-, low-intensity bouts of intermittent running. However, the approach has not previously been applied to regulate exercise intensity during bouts of multidirectional movement. A further novel aspect of the protocol was to reduce training area, with the aim of increasing whole-body decelerations and accelerations, which are characteristic of high-effort actions in team sports, and it was hypothesised this could provide a diverse loading stimulus during change of direction (COD).

1.2.2 Investigative aim

The primary aim was to investigate feasibility and acceptability of brief, diverse HIIT in sedentary females.

A secondary aim was to investigate whether there was an osteogenic effect of exercise on calcaneal bone and background bone metabolism.

1.2.3 Methods and approach

Elevane women aged 35 – 55 were recruited, and trained for 12 weeks with diverse HIIT. Calcaneal bone and bone metabolism were measured at baseline and after 12 weeks, using QUS and biomarkers of bone formation and resorption. Locomotor profile and heart rate during baseline training were quantified using GPS, to characterise physiological demands of exercise in the sedentary basal state.

1.2.4 Outcome and impact

Eight females completed the training and none left the study for reasons of unacceptability of the protocol. Heart rate was elevated during exercise and matched intensity for SSG. No osteogenic effect was observed on calcaneal bone and dynamic coupling of bone formation and resorption markers did not change. However, we did not control menopausal status and confounding effects arising from differences in gondadal steroid hormone milieu could have attenuated potential anabolic effects of training.

Study findings and experimental approach were communicated in poster presentations (see Appendix, A5.1). Principal outcomes were that diverse HIIT was shown to be feasible and highly acceptable in sedentary females, and elicited similar intensity during exercise as SSG. The study provided ‘indication of concept’ and enabled limitations related to inadequacies of exercise prescription to be identified, therefore acute and chronic training studies with modified diverse HIIT were proposed.

1.3 Study 3

‘Characterising Locomotor Profile and Acute Physiological Demands of Diverse Movement-HIIT (DM-HIIT) In Sedentary Women and Female Dancers’

1.3.1 Background and rationale

Diverse HIIT was found to be feasible and acceptable in sedentary females, however physiological demands of training were not fully characterised, and therefore the secondary aim of describing the relationship between activity profile and target outcomes in bone was not achieved.

In order to assess more comprehensively the physiological demands of a single bout of exercise, an acute training study was designed. Sedentary females were recruited, and data provided by female ballet dancers, who trained with the same protocol at baseline in a different study (Study 5), were used to compare responses in two female populations.

The rationale for recruiting from these populations was drawn from evidence that both could benefit from exercise targeted to improve bone health. Sedentary behaviour has been negatively associated with BMD in the hip and femoral regions in females (Chastin, Mandrichenko, Helbostadt, & Skelton, 2014). It was hypothesised, on the basis of observing an osteogenic effect at the hip after low-volume SSG in this population (Study 1), that training with multidirectional football movements in a diverse HIIT format could potentially provide an anabolic stimulus to bone from similar mechanical loading. In ballet dancers, low BMD has been linked to reduced energy availability and menstrual dysfunction (Doyle-Lucas, Akers, & Davy, 2010; Warren et al., 2002), however superimposing SSG to examine its effect on bone is unlikely to be acceptable or feasible within dancers’ training.

It was therefore proposed to characterise responses to an acute bout of training, to provide an estimation of dose-response to diverse exercise in these two populations. The overall aim was to draw on analysis of results to modify protocol design and inform longer-term exercise prescription, using the approach of HIIT training for bone.

1.3.2 Investigative aim

The principal aim was to characterise physiological responses to a single bout of diverse HIIT in two female populations.

1.3.3 Methods and approach

Sedentary premenopausal females (*n* = 15) and female dancers (*n* = 19) in their final year of pre-professional training took part in a single bout of grid training. Calcaneal QUS was used to measure bone, and exercise heart rate and capillary blood lactate concentration to quantify cardiometabolic response to exercise. Locomotor profile was characterised by tri-axial linear and angular accelerometry data, to enable data acquisition during indoor training in dancers. The data were subsequently examined and thresholds created to describe duration of accelerations within different bands, ranging from low- (1- 1.5 g) to high-threshold (> 3 g).

The protocol was modified from the feasibility study (Study 2) to include more high-intensity bouts of movement and increase multidirectional, flighted actions, to enhance impacts and elevate the osteogenic potential of exercise.

1.3.4 Outcome and impact

A single bout of training elicited significant increase in heart rate, achieving thresholds identified for HIIT, and elevated blood lactate concentration. Analysis of acceleration data showed that vertical accelerations predominated within each threshold categorised, in both dancers and sedentary females however, duration of high threshold accelerations >3 g was low (< 2 s).

Whilst outcomes from this study were not widely communicated, and therefore it did not have an external impact, within the scope of this thesis, it demonstrated ‘proof of concept’; i.e., that delivering team sport movements in a focussed bout of diverse exercise replicated physiological demands of SSG.

Furthermore, as duration of vertical accelerations was significantly higher than for all other directions, it was hypothesised that movements that included a flight phase, such as hopping and jumping, could account for this finding. It was therefore inferred that diverse HIIT could promote an osteogenic effect from mechanical strains during landing impacts. However, as duration of high-threshold accelerations was low, and high-intensity accelerations were

considered a proxy indiator of osteogenic potential, it was concluded that in chronic implementation, the protocol should be ramped with the aim of enhancing high-intensity aspects of the locomotor signal, to elevate the potential for an anabolic effect on bone.

1.4 Study 4

'Effects of 12 Weeks Diverse HIIT On Bone: A Randomised Controlled Home Exercise Intervention in Sedentary Premenopausal Females'

1.4.1 Background and rationale

Having demonstrated the feasibility of diverse HIIT in Study 2, and provided insight into physiological demands and acceleration profile during a single bout of training in Study 3, a 12 week study in sedentary females was planned, to examine longer-term effects of training.

As anecdotal evidence from Study 1 suggested females perceived lack of time to be a barrier to exercise, the protocol was adapted for home training, to enable participants to schedule exercise alongside professional and family commitments.

Although acceleration profile showed that high-threshold accelerations were not frequent during one-off diverse HIIT, it was decided not to intensify the stimulus during the initial ramp, in order to accommodate basal sedentary status. It was reasoned that this would reduce the risk of adverse events, associated with unsupervised training at high-intensity in a deconditioned state.

1.4.2 Investigative aim

The principal aim was to investigate whether 12 weeks home exercise, prescribed as 3 x brief diverse HIIT per week, elicited adaptation in bone and bone metabolism in sedentary premenopausal females.

1.4.3 Methods and approach

This was a randomised controlled training study in sedentary, premenopausal females, and 30 participants were recruited and provided data at baseline (*n* = 13 controls; *n* = 17 home exercise [DVD]) and also performed a single bout of diverse HIIT, if allocated to receive exercise. Physiological demands of exercise were characterised by exercise heart rate and

acceleration profile; calcaneal bone was measured using quantitative ultrasound and an early morning fasted blood sample, prior to exercise, was taken to measure background biomarkers of bone formation and resorption. Women in DVD were asked to rate perception of effort after home training.

After 6 weeks, women allocated to diverse HIIT returned and repeated baseline exercise, and acceleration profile was quantified. Home exercise was ramped for the final 6 weeks of home training. All participants repeated baseline data collection procedures after 12 weeks, including QUS and venipuncture for bone turnover markers. DVD performed a third repeat of the quantified diverse HIIT bout implemented at baseline and after 6 weeks; physiological responses to exercise were characterised as at baseline.

1.4.4 Outcome and impact

In total, eight participants did not complete the study (*n* = 5 DVD; *n* = 3 CON), with no pariticipants lost in the exercise group for reasons related to acceptability of the protocol (100% compliance in those completing 12 w). Exercise intensity, characterised by HR_{MEAN} as a percentage of HR_{MAX} during exercise, increased after 12 weeks training, achieving HIIT thresholds, and this was accompanied by a significant training effect on vertical accelerations, which increased in duration across all acceleration bands after 12 weeks training. Rating of effort was significantly higher for supervised training than for home exercise. Calcaneal QUS and biomarkers of bone turnover did not change in either group after 12 weeks, indicating there was no measurable effect on bone of 12 weks diverse HIIT. However, analysis of locomotor profile suggested participants acclimatised to training during the first 6 weeks, and therefore basal sedentary status could have limited osteogenic effects proposed for exercise movements, for example, reducing ground reaction force during impact.

The study was performed in collaboration with Dr Luke Connolly and a team of researchers (See Appendix 4), and he has communicated his findings in a research paper (Connolly et al., 2020) (impact factor: 3.600; 5 citations), to which this author contributed relevant data sets, and assisted with editing and writing.

Overall, this study demonstrated that home training with brief, diverse exercise is an acceptable approach to increase PA in sedentary females.

Evidence that intensity was lower during unsupervised exercise indicated that in future, combining supervision with independent training could be an effective approach to retain intensity of the stimulus provided by exercise.

1.5 Study 5

'Effects of 12 Weeks Supervised Diverse HIIT on Bone Metabolism and Calcaneal QUS in Young Male and Female Ballet Dancers'

1.5.1 Background and rationale

Ballet dancers are at risk of long-term impairment to bone health associated with menstrual dysfunction, which evidence suggests is highly prevalent in this population (Bacchi, Spiazzi, Zendrini, Bonin, & Moghetti, 2013), linked to low body weight and reduced energy availability (Doyle-Lucas et al., 2010) in accordance with REDS phenomena observed in athletes in leanness disciplines. However, the quality of research evidence in ballet dancers, who are a hard to access population, is low, and cross-sectional study designs are more representative (Hincapié & Cassidy, 2010). Within the available body of evidence female dancers are overwhelmingly the focus of research, due to REDS-associated focus on impairment to bone health in female dancers. However, male athletes in disciplines where leanness is an advantage also exhibit lower BMD (Dolan et al., 2012). Furthermore, whereas analysis of activity has shown dance performance elicits similar physiological responses as observed for intermittent team sports (Wyon et al., 2011), the available evidence indicates dancers have lower cardiorespiratory fitness, compared to athletes with similar workloads (Twitchett, Nevill, Angioi, Koutedakis, & Wyon, 2011).

Given evidence that diverse HIIT imposed acute physiological demands (Study 3) and taxed the anaerobic energy pathway, it could provide a feasible approach to address evidence of inadequate fitness in dancers, alongside targeting bone outcomes. Although we observed an osteogenic effect of brief team sports activity (Study 1), volume of pre-professional dancers' training schedules (35 - 40 hours per week) is unlikely to provide opportunity for off-site supplementary exercise with SSG.

To address the lack of longer-term quantitative evidence identified in this population we proposed to conduct a training study in preprofessional male and

female ballet dancers, using the format of diverse HIIT previously examined in sedentary females and sample relevant systemic biomarkers over multiple timepoints,

1.5.2 Investigative aim

The principal aim was to investigate whether there is an osteogenic effect of supplementing ballet training with brief diverse HIIT in male and female dancers. We also proposed to characterise withdrawal from supplemental diverse HIIT and usual training, by conducting a further round of biomarker sample collection, on the dancers' return after the three-week seasonal break in December.

A secondary aim was to examine the effect of diverse HIIT on indicators of cardiometabolic fitness pre- and post- 12 weeks diverse HIIT, superimposed on regular dance activities.

1.5.3 Methods and approach

This was a 12 week training study that adopted convenience sampling; female (*n* = 19) and male (*n* = 10) dancers were allocated to receive supplemental DM-HIIT or trained as usual (*n* = 17 females; *n* = 10 males). Data was collected at baseline and after 12 weeks training and in dancers supplemented with diverse HIIT, detraining effects on biomarkers of bone turnover were examined.

Fasted venipuncture was conducted to measure analytes (gonadal steroid hormones, biomarkers of bone formation and resorption and vitamin D) and bone was measured with calcaneal ultrasound.

To characterise the stimulus, acceleration profile during a diverse bout of exercise was quantified at baseline and after 12 weeks' training, alongside exercise heart rate and capillary blood lactate, to characterise physiological responses to diverse HIIT.

Training was supervised and conducted in the school in small groups twice a week.

1.5.4 Outcome and impact

An osteogenic effect was observed in female dancers supplemented with diverse HIIT, whereby background bone resorption reduced after 12 weeks, and

increased after detraining, however bone formation did not change, and this was attributed speculatively to attenuation of the anabolic stimulus, in association with MD, as there was evidence of gonadal hormone dysregulation in females. In males, biomarker responses to detraining suggested there was a net bone resorption effect from withdrawal of exercise, however this could have also reflected an effect of stopping usual dance training. Vitamin D was significantly reduced in all investigation groups after 12 weeks. Grid training was found highly acceptable and no dancers dropped out of supplemental training; however, acceptability of venipuncture was lower in TAU (n = 11) than in grid supplemented females (n = 19) who all provided blood samples; whereas the same number of males in both groups (n = 9) provided pre- and post- samples.

Findings from this study have been communicated in presentations to an international audience of clinicians, teachers and researchers working in the dance field (Appendix 5.5), and at an international exercise research conference (Appendix 5.6).

The Medical Director of the dance school has used poster information, presenting key outcomes from the study, to educate dancers about their potential risk of low vitamin D and has communicated findings within the dance community, informally and in presentations to peers.

The school continued to train with the protocol, as dancers and staff perceived that it improved dance fitness.

2 Literature review

2.1 Introduction

The purpose of this brief overview of themes relating to bone is to provide some background to what is discussed within chapters describing experimental research.

There, the focus is on discussing research findings in relation to the existing evidence within applied exercise research, and as a consequence, more mechanistic discussion of bone, including bone structure and regulatory mechanisms, are less represented.

The brief overview that follows is an attempt to address this imbalance, and at least touch on important themes that are not substantially discussed within those chapters.

2.2 Bone tissue organisation

Bone is a specialised form of hydrated connective tissue with biphasic material properties. It comprises an inorganic apatite biomineral phase (calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ and an unmineralised organic phase (osteoid), composed of fibres and ground substance, in which Type 1 collagen fibres and osteocalcin, a non-collagenous protein, predominate (Weiner & Traub, 1992). Bone tissue is 50 – 70% mineral phase, 20 – 40% organic matrix, 5 – 10% water and < 3% lipid (Song, 2017), and molecular changes within these phases alter bone properties and therefore bone behaviour, as for example during ageing, when crystallinity increases and bone becomes more brittle (Burr, 2003). As well as providing mechanical rigidity and compressive strength, the mineral phase is a source of calcium and phosphate and therefore bone also contributes to mineral homeostasis, which is regulated by the complementary actions of parathyroid hormone (PTH) and calcitonin, sex hormones, fibroblast growth factor-23 (FGF23) and calcitriol, the bioactive form of vitamin D.

Proceeding from mineralised collagen fibril, the fundamental unit from which bone tissue is formed, through fibrillar array and osteon, and culminating in whole-bone, a hierarchical seven-tier model provides a useful visualisation of bone tissue organisation (Weiner & Wagner, 1998). Whilst this schema helps

explain mechanical properties conferred by the hierarchical arrangement of bone tissue (Abueidda, Sabet, & Jasiuk, 2017), three-dimensional visualisation at nanoscale has refined appreciation of bone structure and revised the nested template to include fractal-like organisation, and the abundance of helical motifs discerned in bone structure (Reznikov, Bilton, Lari, Stevens, & Kröger, 2018). Whole bone remains at the apex of the structural hierarchy within the proposed nine-tiered model, immediately above trabecular and compact bone, which have been extensively characterised in applied research into bone adaptation (Reznikov, Shahar, & Weiner, 2014).

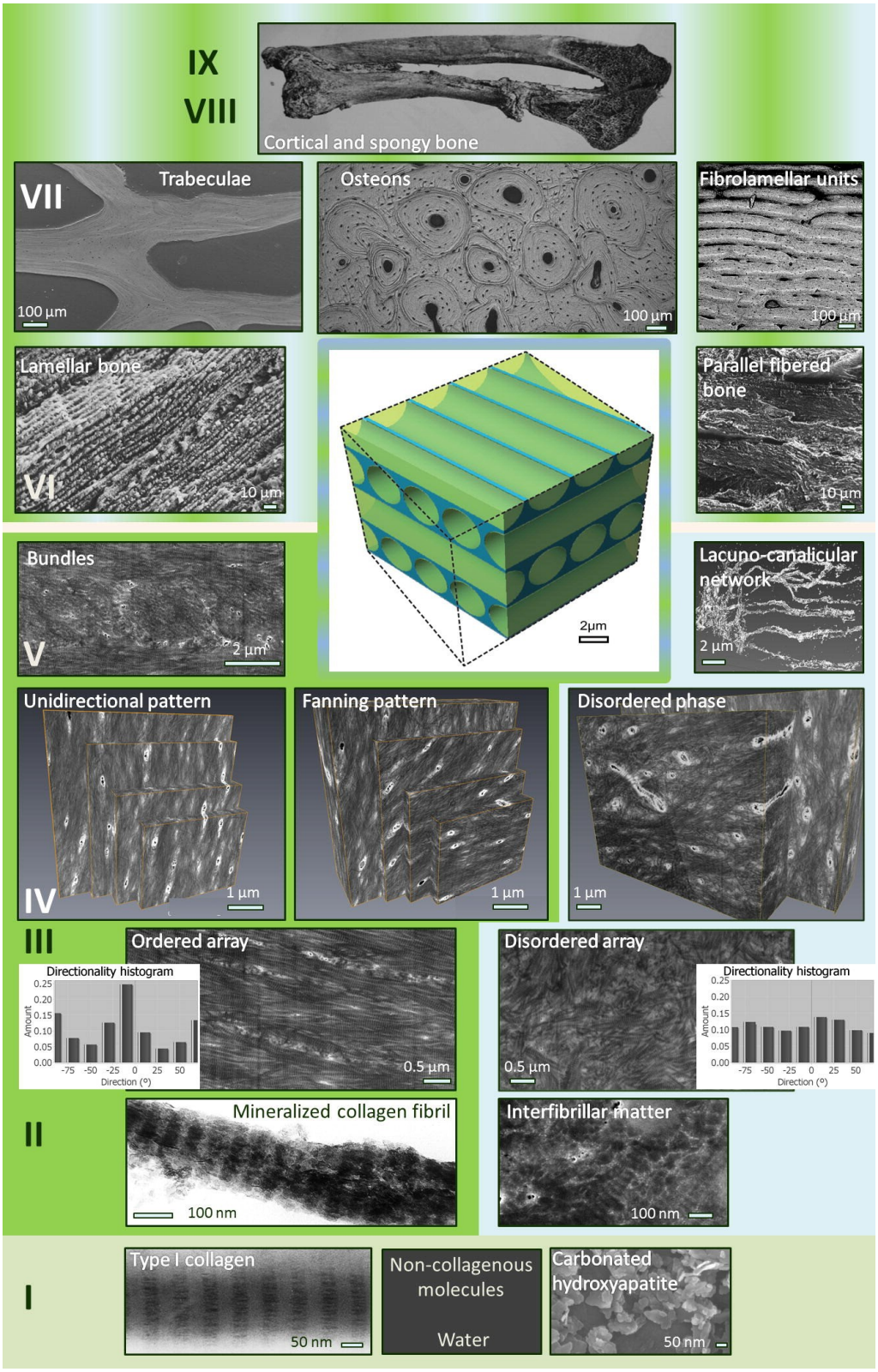


Figure 1 Hierarchical Model of Bone Tissue Organisation

(Reznikov et al., 2014)

In superstructure (above level 5 [V]) bone is composed of lamellae (‘thin layers’), made up of bundles of mineralised collagen fibrils, ~1 – 3 microns in length and aligned in register (Reznikov et al., 2018), an arrangement which accounts for lamellar bone’s anisotropy, i.e., directional difference in mechanical behaviour. At nanoscale, a disordered phase of material has been discerned, which separates and surrounds lamellar bone, and is composed of mineralised matrix, disarrayed Type-1 collagen and non-collagenous organic material, in which cellular components of bone, notably osteocytes and their cellular extensions, reside (Reznikov et al., 2014). Aside from woven bone, a transient material with no discernible alignment of collagen fibres, and fibrolamellar bone, which shares organisational features with tendon and the insertional fibres that locate muscle on bone, lamellar bone comprises the predominant structural element (Currey, 2003).

Descending from the level of whole bone to the compartments immediately beneath, ~80% of bone mass is comprised of cortical bone and of this ~30% is occupied by vascular channels, whereas in trabecular bone, spongy marrow and bone marrow fat comprise ~80% of volume (Ott, 2018), and the latter is a source of adipokine secretomes, which influence the bone microenvironment (Hardouin, Pansini, & Cortet, 2014). Cortical bone, which forms the outer layer of all bones, is denser than trabecular bone and highly resistant to torsional strain, whereas trabecular bone is more elastic, and turnover in the cortical compartment is slower than the trabecular (Kenkre & Bassett, 2018). Trabecular bone, which is found in the vertebral bodies of the axial skeleton, the calcaneus, and at the epiphyses and metaphyses of appendicular long bones, shares an interface with the cortical compartment at the endosteal surface. In macrostructure, trabecular bone resembles a honeycomb-like lattice and is highly (70 – 80%) porous, which confers lightness as well as augmenting compressional stiffness and ductility. The trabecular lattice is comprised of rods and plates of bone (trabeculae), aligned to prevailing load and for optimising mechanical load transference (Oftadeh, Perez-Viloria, Villa-Camacho, Vaziri, & Nazarian, 2015). Whereas at the trabecular level, mechanical behaviour and integrity reflect lamellar properties and orientation, at the fibrillar level this is determined by degree of mineralisation and collagen-matrix interactions (Wu, Isaksson, Ferguson, & Persson, 2018). Bone marrow, housed within trabecular bone, is the niche of haematopoietic stem cells (HSC),

osteolineage cells, notably bone forming osteoblasts, and bone-marrow derived mesenchymal stem cells (MSC), pluripotent stromal cells with the capacity for committal towards either bone, fat or cartilage (Pinho & Frenette, 2019). In adults, red haematopoietic marrow present in early development and childhood is primarily converted to yellow fat marrow, which composes ~7% by volume of total fat (Hardouin et al., 2014). Evidence in younger and older adults that bone marrow fat is inversely related to BMD at an anatomically matched site, and for the whole body (Shen, Chen, Gantz, Punyanitya, et al., 2012), underlines the intimate relationship between structural elements in bone tissue and material properties that influence whole bone behaviour. According to the emerging paradigm of a competitive dynamic between bone formation (osteoblastogenesis) and adipose tissue expansion (adipogenesis) (Shen, Chen, Gantz, Punyanitya, et al., 2012), connectivity between hierarchically organised constituents of bone exceeds simple definition by architectural proximity, and includes the potential for regulatory, permissive, and cell committal functionality.

2.3 Material properties, mechanical behaviour, exercise targets

As a composite tissue the behaviour of bone under applied load is influenced by the material properties of its constituent elements (Rho, Kuhn-Spearing, & Zioupos, 1998): the mineralised phase confers rigidity and stiffness, enabling bone to resist high tensile and compressive forces (Currey, 2003), whilst bone-collagen contributes elasticity and reduces brittleness, conferring resistance to bending stresses, and greater energy absorption prior to failure (Turner & Burr, 1993). These alternating characteristics of rigidity and elasticity, whilst differently expressed in the gross architecture of vertebrate skeletons, are principally deployed to maintain an optimal environment for locomotion, and to resist ensuing strains from repeated locomotor activities (Rubin, 1984), although other aspects of behavioural repertoire, such as habitat utilisation, can influence skeletal morphology (Burr, Ruff, & Johnson, 1989). Architecturally, the skeleton provides protection for the viscera and nervous system, and a semi-rigid scaffold for connective tissue elements and muscle-tendon-bone interactions which, alongside locomotion, enable the expression of highly evolved social and predation behaviours (Pivonka, Park, & Forwood, 2018).

From an applied perspective, bone behaviour expresses complex interactions between the intrinsic material properties of its constituent elements, and the acute and chronic aggregation of environmental influences. Amongst the latter, mechanical signals derived from skeletal loading, which are sensed and transduced by bone, and factors prevailing within systemic and localised milieu, such as gonadal hormone status, adiposity and energy availability, exert their effects within the bone tissue niche, and are somewhat amenable to description through quantifying changes in bone structure and bone metabolism. For example, impact exercise is shown to increase femoral but not spine bone mineral density (BMD) in premenopausal females (Zhao, Zhao, & Zhang, 2014), i.e., results in structural adaptation. Postmenopause, combined interventions with resistance as well as impact appear more effective, particularly to elicit bone adaptation at the lumbar spine (Martyn-St James & Carroll, 2009; Zhao, Zhang, & Zhang, 2017). What may be inferred from this evidence is that bone behaviour under mechanical strain is neither quantal, and therefore not completely determined by dose-response relationships, nor homogeneous in its structural expression. Moreover, bone responses occur within a biological context, which influences regulatory processes, cell committal and signalling cascades within the bone milieu, as demonstrated by this example of contrasting responses to skeletal loading in the oestrogen-replete and insufficient state.

An example of this is provided by evidence in the obese and overweight phenotype, which according to the mechanical model of body mass-dependent effects of skeletal loading, is at a pro-bone advantage. However, evidence of impairment in bone architecture in obese compared with non-obese postmenopausal females (Sornay-Rendu, Boutroy, Vilayphiou, Claustrat, & Chapurlat, 2013), and of increased pelvic fracture risk in obese compared with non-obese children (Kim, Hsieh, Soni, Zayzafoon, & Allison, 2013), suggests the relationship between body weight and bone strength may not be unidirectional. In fact, there is complex interplay between adiposity and bone, in which inflammatory processes, linked to a tendency for reduced insulin sensitivity and increased type 2 diabetes (TD2) in the obese phenotype. This has been demonstrated by evidence of increased fracture risk associated with T2D in obesity, where, paradoxically, the mechanical advantage towards higher skeletal loading conferred by body weight is opposed by greater systemic

inflammation arising from reduced insulin sensitivity (Ma, Tonks, Center, Samocha-Bonet, & Greenfield, 2018).

Within the definition of material properties, strength is described as the capacity to withstand an applied load without incurring a plastic (i.e. non-elastic) deformation, up to the point of structural failure (yield point). In bone, stiffness is represented by the linear part of the stress-strain curve, where bone behaviour is elastic and deformation is recoverable, up to the point of fracture, which corresponds to ultimate bone strength. Toughness is described by area under the stress-strain curve until the yield point and corresponds to the energy absorbed before tissue failure (bone fracture) (Ammann & Rizzoli, 2003). The material properties considered most influential on skeletal fragility are strength, capacity to absorb energy (fracture toughness) and resistance to fatigue (fatigue strength) (Hernandez & van der Meulen, 2017). Unlike strength, which mechanically is defined by load to failure, i.e., a maximal, one-off loading event, as potentially represented by a fall, resistance to fatigue describes an arguably more relevant, functional bone property. It encompasses the capacity to withstand long-term, cyclical loading, and therefore is more representative of the mechanical strain environment to which the skeleton is exposed during habitual physical activity (PA). Microdamage in bone is recognised to contribute to skeletal fragility in osteoporosis (Burr, 2003), and whereas bone architecture has been shown to be impaired in association with gonadotropic hormone dysregulation in young female athletes (Ackerman et al., 2011), it is positively associated with indices of skeletal loading in young adults (Popp et al., 2019). Evidently, the influence of PA and exercise on material properties of bone is complex, and not fully captured by the binary scale of ‘weakness-strength’.

Very broadly, strength, and strengthening bone, have been primary targets within interventional approaches to exercise and bone (Nikander et al., 2010), particularly to target osteoporosis, a clinical entity comprised of ‘compromised bone strength’ (Bartl & Bartl, 2019), a definition which underlines how strength has tended to be perceived as the principal component in skeletal health. Regimens targeting improvement in bone have often pursued this as an outcome (Nikander et al., 2010), yet it is increasingly appreciated that other properties, such as geometry and prevailing architectural features of bone

tissue, significantly influence whole-bone behaviour, and moreover, appear amenable to exercise-induced adaptation (Harding & Beck, 2017).

For example, whereas impact exercise is recognised to increase BMD (Zhao et al., 2014), impacts from jumping are also demonstrated to improve bone geometry (Vainionpää et al., 2007). A longitudinal study in adolescent males reported superior BMC in those who played football compared with swimmers and cyclists, with evidence of significant, positive adaptation in trabecular bone score (TBS), which increased by 4.3% and 4.2% in footballers, compared with swimmers and cyclists, respectively (Vlachopoulos et al., 2017). This reenforces that odd and diverse impact sports engender adaptation beyond increases in BMD. Interestingly, whereas research in ballet dancers has investigated, and frequently confirmed, low BMD in association with low energy availability and menstrual dysfunction (Doyle-Lucas et al., 2010; Warren et al., 2003), using 3D-DXA to measure bone, ballet dancers exhibited significant adaptation at weight-bearing sites within the femur, in both cortical and trabecular phases, despite low energy conditions, and were superior to controls (Freitas et al., 2019). Perhaps, as resolution of other properties in bone becomes more accessible, osteogenic effects of long-term exercise will also be evaluated in terms of adaptation in bone geometry and architecture, and their contribution to maintenance of skeletal health.

2.4 Cellular components of bone

From the perspective of applied research into bone, osteoblasts and osteoclasts, which within bone tissue are the effectors of formation and resorption, are the cellular components which are perhaps of most interest, whereas the role of mechanosensation is attributed to the osteocyte (Bonewald & Johnson, 2008). In global terms, applying exercise to improve bone health can be thought of as attempting to tilt the dynamics of formation-resorption in favour of the osteoblast. Therefore, by uncovering mechanisms that underlie exercise effects at a cellular level, there is the potential to influence applied practice and leverage a pro-bone response.

A key area of research focus has been the relationship of adipose tissue and bone, and factors influencing the fate of cells descended from the MSCs within the bone marrow niche. Transcriptional factors regulating cell differentiation have been described within fundamental research into cell

lineage (Komori, 2006). Whilst this is not the focus of an applied interventional approach, evidence from cell culture and animal studies has helped identify mechanisms underlying the observed improvement in bone from exercise, particularly in inclining MSC committal towards an osteoblast endpoint. In a murine model, endurance exercise induced expansion of MSCs, enhanced committal to osteoblastogenesis and a lowering of adipogenic inclination (Marędziak, Śmieszek, Chrząstek, Basinska, & Marycz, 2015). It is proposed that the pathway of influence that exercise opens towards osteoblast committal is mediated by Wnt signalling (Yuan et al., 2016). Evidence also suggests that mechanical signals associated with exercise directly promote differentiation towards an osteogenic endpoint, as well as acting as a repressor to adipogenesis, and may directly regulate adipose tissue within the marrow (Pagnotti & Styner, 2016). Emerging evidence demonstrating the intimate and interconnected fates of bone and fat, suggests that exercise not only promotes osteogenic effects via increased osteoblast formation, but also by influencing the ‘cellular hardware’ within the marrow niche.

Osteoclasts have a different descent from osteoblasts and are derived from the macrophage lineage of haematopoietic stem cells. Osteoclastogenesis is initiated by RANKL binding to the receptor RANK on the surface of osteoprogenitor cells, whereas it is inhibited by osteoprotegerin (OPG), which can bind RANK and act as a decoy (Boyce & Xing, 2008). As both OPG and RANKL are expressed by osteoblasts, their role is crucial in initiating or blocking the cascade that leads to resorption. Therefore, in essential terms, exercise expresses effects on bone via the RANKL/RANK/OPG pathway (Tobeiha, Moghadasian, Amin, & Jafarnejad, 2020). However, characterising OPG as a way of examining potential dose-response effects of exercise on this pathway has provided conflicting evidence. For example, whereas plyometric jumping elevated OPG in younger male participants (Kish, Mezil, Ward, Klentrou, & Falk, 2015), this effect was not seen in females, although RANKL did reduce (Dekker et al., 2017). It is also proposed that mode-dependent effects (e.g. for brief impact versus endurance) of exercise may be characterised according to responses along this pathway (Tyrovola & Odont, 2015), and certainly the RANKL/RANK/OPG pathway provides a potential candidate approach to describe dose-response effects of exercise on bone, at higher biological resolution.

2.5 Mechanotransduction and bone

Identifying potential pathways involved in orchestrating adaptive responses in bone, the so-called 'black box' of mechanoreception and transduction (Pearson & Lieberman, 2004), has been the focus of research using animal models (Meakin, Price, & Lanyon, 2014), and greater understanding of how loading is sensed and integrated in bone is likely to be important in increasing the efficacy of applied interventions to improve bone health. Landmark studies have demonstrated in vitro that whilst bone cells respond rapidly to mechanical loading (Lanyon & Rubin, 1984) saturation occurs quickly (Turner & Pavalko, 1998). Inclusion of recovery periods and intermittence in loading cycles appears to maximise the osteogenic effects of loading, via restoration of mechanosensitivity (Robling, Burr, & Turner, 2001; Robling, Hinant, Burr, & Turner, 2002).

Applying load dynamically, as opposed to statically, increases bone formation in isolated animal models (Lanyon & Rubin, 1984) and under laboratory conditions, if all other parameters are controlled, a dose-response relationship has been demonstrated between peak strain magnitude and bone formation (Gross, Edwards, Mcleod, & Rubin, 1997; Turner, Akhter, Raab, Kimmel, & Recker, 1991). In vivo findings support the association between strain magnitude and bone formation, with evidence of periosteal deposition correlated with areas of high-strain application during exercise (Judex, Gross, & Zernicke, 1997; Judex & Zernicke, 2000), however more recent experimental evidence with animals during treadmill loading has challenged assumptions that site and direction of high strain are inevitably closely correlated (Wallace et al., 2014). Whilst caution is necessary in applying in vitro findings to the whole organism, and in translating evidence from animal models to human subjects, mechanistic evidence supports the validity of applying intermittence and dynamic loading within experimental design as a deliberate strategy to increase the efficacy of exercise on bone metabolism. This could explain evidence of higher BMD for longterm athlete participation in exercise where intermittent high-intensity is characteristic of activity patterns (Ubago-Guisado, Gómez-Cabello, Sánchez-Sánchez, García-Unanue, & Gallardo, 2015).

Candidate theories of mechano-sensation and transduction, which implicate mechanical deformation of the perilacunar bone matrix and fluid flow

within the cellular bone environment, offer an additional body of mechanistic evidence which may inform translational study design. It has been proposed that force-induced deformation within the ultrastructural bone environment is integrally coupled with mechanotransduction, via complex intracellular signalling processes involving osteocytes, osteoblasts and bone-marrow stromal cells and the Wnt/βcatenin pathway (Bonewald & Johnson, 2008; Turner, Forwood, & Otter, 1994; Weinbaum, Guo, & You, 2001). It therefore is plausible that the osteogenic potential of exercise could be enhanced according to these mechanisms. For example, utilising diverse movements deliberately to induce high strain rates in bone, with the aim of inducing greater or more frequent deformations of skeletal bone matrix and its structures, may generate greater shear stress and affect the rate and orientation of fluid flow across bone cells, increasing the intracellular signalling implicated in orchestrating bone responses to loading (Raggatt & Partridge, 2010). The candidate theories to explain mechanotransduction, i.e., whether deformation is predominantly perilacunar or canalicular, are in agreement that direction (in deformation or flow) and rate of application (from bending or shear stress resultant) are key factors in bone response. Extrapolating from this more fundamental evidence, exercise modalities which emphasise diverse loading, for example, during whole-body accelerations and decelerations in non-uniform movements, may induce an osteogenic effect from high strain rates and high-magnitude forces, applied in a non-linear manner across bone tissue. Cross-sectional findings from athletes exposed to diverse and non-usual loading of bone architecture and femoral neck density comparable to athletes participating in high-impact activities further supports this (Narra, Nikander, Viik, Hyttinen, & Sievänen, 2013; Nikander, Sievänen, Heinonen, & Kannus, 2005).

These principles may also offer a possible mechanistic explanation for the observational and training study findings previously cited of high BMD in athletes who participated in football (Fredericson et al., 2007), and regional increases in hip and femoral BMD after recreational football training (Jackman et al., 2013; Krustrup, Christensen, et al., 2010). The evidence supporting football as a superior form of exercise in terms of its osteogenic effects may in part be attributed to its episodic pattern of activity and recovery, i.e. as a modality of exercise involving high forces characterised by intermittence, which mechanistic findings suggest favour bone adaptation.

Elsewhere, evidence suggests that utilising a high impact approach in exercise intervention is associated with regional increases in bone mineral density at the femoral neck (Kato et al., 2006; Niu et al., 2010; Niu, Feng, Jiang, & Zhang, 2014) and in the lumbar spine and femur (Vainionpaa et al., 2007); and that this is intensity dependent (Ahola, Korpelainen, Vainionpää, & Jämsä, 2010; Vainionpää et al., 2006). It has been proposed (Helge et al., 2010) that high strain rates and ground reaction forces associated with football movements may account for observational evidence of increased bone mass in both female (Söderman, Bergström, Lorentzon, & Alfredson, 2000) and male players (Fredericson et al., 2007), and participation in ball sports during adolescence, when bone turnover is rapid, appears to offer protection against future stress fracture in runners (Fredericson, Bergman, Hoffman, & Dillingham, 1995). Cross-sectional evidence in gymnasts of greater bone mineral density (Helge & Kanstrup, 2002) alongside bone adaptation towards greater resistance to axial loading and fracture (Dowthwaite, Rosenbaum, & Scerpella, 2011) supports anabolic effects for these exercise modalities. The evidence suggests that sports featuring brief, high-intensity actions, involving impacts and whole-body accelerations, promote positive adaptations in bone, which may be mechanistically linked to the high strain rates and ground reaction forces associated with participation.

Although the evidence of an osteogenic effect of diverse impact activities, particularly for longterm participation in elite and recreational athlete populations, is compelling, specific mechanical factors governing response are still not fully elucidated. In order to examine the relationship between biomechanical intensity and skeletal response, Turner and Robling proposed an osteogenic index (OI) comprised of three variables: peak vertical ground reaction force (GRF) as a multiplier of BW and indicator of intensity, number of loading cycles during a bout of activity, and number of sessions (Turner & Robling, 2003); the latter two variables represent volume of exposure to the stimulus. Refined in definition as:

$$\mathrm{OI} = I * \mathrm{LN}(N + 1)$$

where I is the intensity of exercise, LN is the natural logarithm, and N is the number of loading cycles produced by the exercise, this approach has facilitated more meaningful comparison between regimens, enabling aspects of

the mechanical signal to be decomposed into factors describing volume and intensity characteristics, with greater potential to elucidate their relationship with bone responses observed over time.

For example, a study in inactive premenopausal women applied the OI approach to examine the effect of 8 weeks' activity exposure, comprised of three weekly bouts ('dose') of either resistance training (RT), aerobic running or combined RT and aerobic training ('stimulus'), and used pQCT to measure the outcome in bone ('response'). The study reported a significant increase in BMC was demonstrated at the most distal tibial site in the combined exercise group, whose activities yielded the highest, mean weekly OI (38.5 AU), for aerobic training (OI: 22.4 AU) and resistance only (OI: 19.0) (Lester et al., 2009).

Whilst this suggests a positive dose-response relationship for an osteogenic effect of exercise combining externally applied resistance with whole body impacts, at least at sites in close proximity to GRF transients, prospective examination of the relationship between OI and osteogenic response have quantified more precisely magnitude of change for a given OI exposure, and given an indication of adaptive timeframe. For example, a 12 month controlled study in premenopausal females titrated loading at the radius (high vs low strain magnitude; high vs low strain rate) in four experimental arms. The study reported an increase of 2.7 ±2.1% in distal radius BMC for low- compared with an increase of 3.4 ±2.2% for high- magnitude loading, whereas in controls BMC reduced at this site by 1.3 ±2.7% (Troy et al., 2020). Whilst the magnitude of bone response was similar to that observed in young females during a 6 month pilot study, which applied the same approach to upper limb loading (Troy, Edwards, Bhatia, & Bareither, 2013), the authors found that loading dose accounted for only 12% of the variance in bone adaptation observed (Troy et al., 2020).

There are a number of implications raised by the findings of Troy and colleagues: firstly, they provide evidence of a positive dose-response relationship for high strain magnitude and high rate loading, in a region specific manner for an upper limb long bone, whereby a small (~3 %) but significant increase in BMC was found; secondly, this effect may be observed in as little as 6 months, and increases in a duration dependent manner after a further 6

months loading exposure. However, as dose explained only a relatively small percentage of variance in bone outcomes, it may be speculated that other factors, such as biological and tissue-specific phenotypic differences between participants, accounted for the observed heterogeneity in osteogenic response. Two further examples of exercise interventions for bone health serve to illustrate this proposal: whereas 10 maximal jumps three times per week have been shown to increase BMD in young women (Kato et al., 2006), 12 weeks bilateral hopping at the same weekly frequency did not increase biochemical markers of bone turnover in elderly (~72 y) males (Rantalainen et al., 2011). Despite utilising OI to characterise the exercise stimulus, and prescribe hopping at maximal (to the individual) GRF, the authors concluded that altered mechanosensitivity, leading to lower responsiveness to the stimulus, could account for the lack of observed response in bone turnover markers in these elder males, particularly as an elevation in bone anabolic response was previously observed in young males exposed to the exercise protocol (Rantalainen et al., 2009).

Overall, the evidence from interventions of both longer and shorter duration suggests that even at optimal prescription, according to OI evaluation of exercise characteristics, osteogenic response to exercise demonstrates considerable variation. Furthermore, as GRF has been shown to vary depending on how movements are performed during pre-set choreography (Santos-Rocha, Oliveira, & Veloso, 2006), using methods to profile movement characteristics, such as accelerometry and GPS, alongside evaluation of the exercise signal according to volume, duration and intensity, could enhance resolution of dose-response characteristics for exercise protocols targeting an osteogenic outcome.

2.6 Hormone actions

Numerous hormones have been identified to play an important role in bone maintenance and metabolism. Gonadal sex steroid hormones, such as oestrogen, are involved in bone maintenance and permissively influence bone responses to loading. Oestradiol exerts its effect directly via ER-α and ER-β receptor activity in osteoblasts and osteocytes (Lee, Jessop, Suswillo, Zaman, & Lanyon, 2003), and less directly by influencing marrow stromal cells towards pro-oestogenic committal and osteoblastogenesis (Okazaki et al., 2002). If

circulating levels of oestrogen are reduced, as observed during peri- and post-menopause and in states of low energy availability, such as anorexia nervosa, or in female athletes failing to meet energy demands (STAND, 2007), bone responses to loading are impaired and bone metabolism is dysregulated (Bidwell, Alvarez, Hood, & Childress, 2013). Targetted exercise therapy for bone health has been examined in pre- and postmenopausal women, and provide evidence that regimens utilising high-impacts, odd-impact loading and eliciting high-magnitude ground reaction forces (GRFs) are more successful (Kohrt, Ehsani, & Birge JR, 1997; Martyn-St James & Carroll, 2009; Martyn-St James & Carroll, 2010) than those lacking these exercise features.

As exercise effects on bone appear to be potentiated by exogenous administration of oestrogen (Cheng, Sipilä, Taaffe, Puolakka, & Suominen, 2002; SIPILÄ et al., 2001; Taaffe et al., 2005), exercise interventions in populations where circulating oestrogen levels may be compromised, such as in peri- and postmenopause, and female athletes with negative energy balance, offer the potential to examine potential interactions between energy status and gonadotropic hormone profile and responses to exercise in bone.

Adequacy of vitamin D, a hormonal mediator of calcium homeostasis via intestinal $1,25(OH)_2$ signalling, is critical in bone health and correction of vitamin D deficiency has been recommended to maintain skeletal health and reduce fracture risk in older adults (Bischoff-Ferrari et al., 2005) however, more recently, general population evidence not shown a consistent positive association between vitamin D status and BMD (Bischoff-Ferrari, 2009; Marwaha et al., 2005), and a positive effect of vitamin D supplementation on BMD, at least in older adults, is not fully supported in the available evidence (Reid, Bolland, & Grey, 2014).

3 General methods

3.1 Introduction

This section presents the rationale for methods used to gather data, in accordance with principal investigative aims outlined for each study. Advantages and disadvantages are considered, alongside their implication for results, as well as interventional context, particularly ecological constraints imposed by location and population, and how these affect choices in methods and investigative approach. Procedures for methodologies discussed in the present section are also described, and referenced within methods sections of each study.

3.2 Anthropometry and body composition

At the level of the individual, anthropometry is used to characterise risk in relation to population-derived values, or to assess an individual's response to an intervention (WHO, 1995). Measurement of height and weight is used to calculated body mass index (BMI), which normalises weight to height and has been experimentally validated as an index of adiposity using extensive population data (Heymsfield, Peterson, Thomas, Heo, & Schuna Jr, 2016; Hood et al., 2019). However, BMI has been demonstrated to be only a moderate predictor of percentage body fat (Jackson et al., 2002), with age and gender accounting for a significant percentage of variance in BF (Gallagher et al., 1996). In females, body fat and percentage fat have consistently been found to be greater than in males, and to increase in both sexes with age (Jackson et al., 2002; Meeuwsen, Horgan, & Elia, 2010).

Fat mass (FM), lean tissue mass (LTM) and bone mineral content (BMC) are also measured using dual x-ray absorptiometry (DXA), in what is referred to as a three-compartment model (Ellis, 2000; Roubenoff, Kehayias, Dawson-Hughes, & Heymsfield, 1993). DXA is considered a low-dose x-ray test to assess body composition, delivering an effective radiation dose of 5 – 30 microsieverts for a whole-body scan (Kendler et al., 2013). Measurement of visceral adipose tissue (VAT) using DXA has shown high agreement (r^2 = 0.957) against computed tomography (CT), considered a gold standard for VAT measurement, whereas agreement for waist circumference (WC) with CT was

demonstrated to be lower (r^2 = 0.686) (Kaul et al., 2012), although this is a practical option in an applied setting (Cornier et al., 2011).

Precision for short-term assessment of body composition by DXA, expressed as coefficient of variation (CoV) for the measurement result, has been reported as ~1.0% for LTM and 0.8 – 2.7% for FM, and is greater for whole-body than for regional measurement (Toombs et al., 2012). DXA is reported to be highly sensitive and reliable in detecting regional changes in fat distribution in longer-term (6 – 12 months) scenarios (Kendler et al., 2013), however whilst low precision error (0.7 – 2.6%) was reported for DXA measurement of TLM, FM and BMC, precision error for VAT was observed to increase with obesity (Meredith-Jones, Haszard, Stanger, & Taylor, 2018). This requires consideration in interventions in inactive female populations, in whom adipose tissue may be greater, and in larger broader subjects, such as athletes, who may exceed scanning dimensions (Silva, Heymsfield, & Sardinha, 2013).

A study that examined the effect of fasted state and physical activity (PA) on serial examination of body composition reported least significant change (LSC) for DXA in females as 290 g for total mass (TM), 410 g for TLM and 350 g for FM (Nana, Slater, Hopkins, & Burke, 2013). In order to limit confounding effects of so-called lifestyle factors, such as recency of physical activity and food consumption, time of day and clothing should be standardised for DXA measurement of body composition where detecting differences of less than 2 kg in total BM is required (Hangartner, Warner, Braillon, Jankowski, & Shepherd, 2013).

Magnetic resonance imaging (MRI) is also used to measure visceral (VAT) and subcutaneous (SAT) adipose tissue volumes. MRI and CT estimates of interstitial adipose tissue and SAT have been shown to be highly correlated (r^2 = 0.98 – 0.99) with ‘true’ cadaver values (Mitsiopoulos et al., 1998), and MRI has the advantage of not exposing subjects to ionising radiation, unlike CT. A positive relationship has been proposed between central adiposity and systemic inflammation (Wajchenberg, 2000) and is supported by cross-sectional population findings of a negative relationship between VAT and trabecular and cortical bone (Zhang, Peterson, Su, & Wang, 2015). If accessible, MRI presents a well validated method for assessment of the relationship between VAT and SAT and bone in an interventional setting.

Without access to image acquisition to determine body composition, direct measurement of hip (HC) and waist (WC) circumference may be undertaken to assess the effect of an intervention on body composition. This approach is useful in an applied setting, where participants cannot travel off site to access DXA or MRI, and is a low-cost method which enables rapid data acquisition in a situation where there are time constraints on access to participants. Furthermore, the procedure is simple to perform and of minimal inconvenience to participants.

Although waist circumference (WC) has been shown to be a strong predictor of truncal adiposity (Ashwell, Cole, & Dixon, 1996; Ehrampoush et al., 2017) measurement site has differed between investigators, however trunk fat mass and WC were found to be highly correlated (r^2 = 0.77 - 0.80) and reproducibility was high (r = 0.996 – 0.999) in a study which compared four approaches to anatomical site for measurement of WC (Wang et al., 2003).

Lower reliability for WC has been demonstrated in obese compared with lean subjects (Nordhamn et al., 2000), from which it may be inferred that in phenotypically lean subjects, such as dancers, in whom anatomical bony landmarks are readily accessible to visual inspection and palpation, measurement of WC may be an acceptable alternative to imaging in a field-based setting. In Study 3 and 4, WC and HC measurements were performed according to the approach recommended by the World Health Organisation (WHO) (WHO (Consultation, 2008)).

In a large (*n* = 218, 212) population study in females, intra-observer reliability for WC was reported to be high (Intraclass correlation coefficient [ICC]: 0.983, 95% CI: 0.990) (Chen, Lear, Gao, Frohlich, & Birmingham, 2001), which suggests that to increase reliability the same examiner, where possible, should undertake repeated measurements of participants.

A further important consideration, when working with adolescent participants, is the effect of relative maturity, and the substantial variation in timing of onset and progression observed between individuals. Non-invasive approaches, which draw on anthropometric indices that are easily accessible, such as sitting height and leg length, offer a potential solution to this issue by applying sex-specific regression equations to predict onset of maturity and age

of peak height velocity (APHV). Whereas systematic bias has been reported, such that predicted APHV may be underestimated in an aesthetic athlete cohort with characteristically later onset of maturation and PHV (Malina et al., 2006), commonly applied equations have been recently redeveloped with the aim of addressing issues arising from over-fitting, and to provide recalibration of predictors in further populations (Moore et al., 2015). Whilst Moore et al., have provided simplified equations that do not require seated posture height to be measured, and the redeveloped equations are suggested to produce a more accurate fit across a range of external samples, the authors cautioned that in late maturers, prediction error was 2-3 times greater in boys and 6-7 times greater in girls (Moore et al., 2015). Therefore the issue of maturity prediction accuracy in young athletes, particularly if maturation onset may be delayed (and offer a competitive advantage in being so), remains pertinent, highlighting the need for specific data acquisition in performance populations. Maturation may also be estimated in a clinical setting using Maturity Rating (SMR), which applies a scale (derived from observational data) to assess developmental stage, according to description of external genitalia (males) and breast development (females) and pubic hair (both sexes). A recent review, whilst concluding that young people found self-assessment to be acceptable, followed in preference by clinical assessment by a practitioner of the same sex, highlighted the disparity (43 - 81%) between these options (Walker, Smith, Davies, Inskip, & Baird, 2020), which suggests that in settings where self-report is the only approach sanctioned, results are unlikely to demonstrate agreement with external clinical evaluation.

3.2.1 Measurement of body mass

Participant body mass (kg) was measured on arrival in a fasted state using a digital column scale (Seca, SEC-170, Seca, Hamburg, Germany). In an applied setting, a portable weighing scale was used (Salter Ultra Accuracy Scales, Salter, UK). Prior to measurement the measuring device was checked to ensure it was in a level position.

After explaining the procedure, the participant was requested to remove outer clothing and shoes and heavy objects (such as mobile phone, jewellery, wallet or purse) from pockets. The scale was switched on and when 0.00 was displayed, the participant was instructed to stand on the scale, with even

distribution of their body mass between both feet, looking straight ahead and with their arms relaxed at the side of the body. When the display showed a steady value, body mass was recorded to the nearest 0.1 kg and the participant was asked to step off the scale and the procedure was repeated three times, or until three consecutive readings were obtained within 0.1 kg of each other. An average was calculated from three consecutive values.

Participants were requested to wear the same clothing as at baseline during exit procedures to standardise measurement conditions.

3.2.2 Measurement of height

Height was measured using a stadiometer (Seca stadiometer SEC-225; Seca, Hamburg, Germany). Prior to measurement the procedure was explained to the participant, who was asked to remove shoes and outer clothing and, if necessary, to roll up trousers to enable heel and foot position against the back of the stadiometer plate to be visually inspected. If appropriate for removal, it was requested that headwear or scarves were taken off, where this was not possible a rod was placed horizontally across the top of the head and its dimension subtracted from the measured height. The participant was asked to stand on the stadiometer facing directly forwards, as tall and straight as possible, with arms relaxed at the side of the body. Feet were positioned under the hips to aid balance and head position was checked to ensure a midline position, tragus aligned horizontally with the inferior orbital margin (Frankfort position). The participant was asked to take a deep breath in and hold this position looking ahead, while the stadiometer plate was brought down to rest on the crown of the head. After checking that knees and spine were extended (avoiding postural slouching), height was recorded to the nearest mm. Three consecutive measurements were taken according to this procedure; if values were more than 2 mm different, the procedure was repeated until three readings that fell within this margin of difference were obtained.

3.2.3 Calculation of Body Mass Index BMI)

BMI was calculated by dividing body mass (kg) by [height (m)]^2.

3.2.4 Measurement of hip and waist circumference

Participant waist (WC) and hip (HC) circumference were measured in a fasted condition, using the protocol recommended by WHO for determining

anatomical measurement site (Consultation, 2008). Using a non-stretch measuring tape directly against the skin, measurement of the waist was undertaken at the end of expiration with the tape positioned mid-way between the lower margin of the most inferior rib and the top of the iliac crest, and hip measurement was obtained at the widest portion of the buttocks. The tape was applied snugly but without constricting, aligned parallel with the floor, and the participant was asked to adopt an upright posture, looking forward and avoiding postural slouching, with weight evenly distributed between the feet. Measurement for WC and HC was recorded to the nearest 0.1 cm. The participant was then instructed to relax and after 10 s to re-adopt measurement posture as described. Three consecutive measurements were performed to within 0.2 cm of the first value obtained, and an average calculated for WC and HC. Waist to hip ratio (W:H, Arbitrary Units [AU]) was calculated by dividing average WC by HC (cm).

Measurement of WC and HC was undertaken by the same experienced examiner during pre- and post-intervention data acquisition.

3.2.5 Measurement of blood pressure and heart rate at rest

Prior to measurement of heart rate (HR) and blood pressure (BP), the procedure was explained, and it was requested that any restrictive clothing, particularly around the arms and neck, was loosened. The participant was then asked to sit quietly for five minutes, legs uncrossed. An automated sphygmomanometer cuff (M7; OMRON, Lake Forest, IL, USA) of an appropriate size to cover 80% of the upper arm was positioned at the level of the participant's heart and rotated so that the device marker was over the brachial artery, as shown in manufacturer instructions on the cuff. The arm was rested on a pillow placed on the supporting arm of the chair during measurement, which was performed on the dominant arm. The participant was asked not to speak during measurement procedures and was instructed to rest with the cuff deflated for three minutes between consecutive measurements. An average value for systolic (SP) and diastolic (DP) blood pressure and heart rate at rest were calculated from three consecutive measurements.

Mean arterial pressure (MAP) was estimated according to the following formula:

- MAP = DP + 1/3(SP – DP)

3.2.6 Dual-Energy X-Ray Absorptiometry (DXA)

A DXA scan (GE Lunar Prodigy, GE Healthcare, Bedford, UK) to measure bone and body composition in regions of interest was performed as described below. Quality assurance (QA) was carried out on the scanner using the block phantom for the scanner to ensure accuracy and assess calibration before each session of participant scanning. Additional QA tests using the aluminium spine phantom supplied by the manufacturer were undertaken on a weekly basis during the study period, to ensure consistency and accuracy in calibration of the scanner.

The body was segmented in accordance with standard procedures to evaluate body composition in regions of interest, and the scans performed by suitably qualified and experienced researchers.

The effective radiation dose for each DXA scan performed was calculated as 7.8 μSv per scan, a level considered to present a 'trivial risk', in the context of reference values for the daily background level of radiation in the UK of 6-20 μSv, depending on locality (Baim et al., 2005).

Procedure After QA procedures were completed, the procedure was explained to the participant and the operator checked that, as requested prior to attending for DXA, clothing was free of metal, jewellery and piercings were removed, and pockets were empty. A gown was provided if metal in clothing or radio opaque buttons, which could create artifacts, were found or if clothing were unusually restrictive and might compress soft tissue, which could affect BMD measurement. Height and weight were then measured (see sections 3.2.1 and 3.2.2) for data entry into the scanner so that optimal scan mode would be selected automatically during the procedure.

To perform a lumbar spine scan, the participant was positioned in supine lying, legs elevated on a block, and pillows were used to support axial cervical spine alignment if required. Anterior superior iliac spines (ASIS) were aligned in the transverse and frontal planes to eliminate lumbopelvic rotation, and the participant's median sagittal plane (MSP) aligned with the longitudinal axis of the scanner bed. The laser cross was placed over the MSP at the mid-point between the ASIS and iliac crests and the scan initiated from the inferior aspect

of the L5 body, to include from mid-L5 to mid-T12 within the scan. The scan operator observed the practice of ‘counting up’ from L5, as prevalence of a transitional lumbar vertebra can be as high as 35.6 % in the general population (Apazidis, Ricart, Diefenbach, & Spivak, 2011), and this approach helps identify correct vertebrae for assessment. During the procedure, the participant was visually checked to ensure optimal position on the bed and the scan was paused and the participant’s posture re-adjusted, if necessary.

To scan the hip, the hip positioner was placed between the participant’s feet in supine lying and the non-dominant hip abducted 15° and internally rotated 25°, checking that rotation originated in the femur and was not a foot driven action. The foot was maintained in contact with the hip positioner and the longitudinal axis of the femur aligned with the edge of the scanner bed. The scan was initiated by placing the laser cross hairs 5 cm below the greater trochanter and scanning completed once a distance of 3-5 cm above the greater trochanter was visualised.

To perform a whole-body scan, in supine lying the participant MSP was aligned perpendicularly to the scanner bed leaving 5 cm clearance between the top of the head and the edge of the scanner bed. The participant was positioned in hip abduction but with the toes of one foot maintaining contact with the other, knees and arms extended, either palms down or laterally rotated, with a space between the arms and the side of the body. To initiate the scan, the horizontal line above the shoulders was positioned underneath the chin and the vertical shoulder lines aligned with the glenoid fossa. The paravertebral lines were moved close to the spine and aligned with existing curvature of the spine if present. The smaller horizontal line was positioned at the T12 – L1 level. The horizontals above the pelvis were positioned above the iliac crests and the angled lines inferior to this aligned so that they bisected the femoral neck on each side. The vertical between the legs was placed between the feet and the lateral lines positioned to include lateral soft tissue as much as possible, without including digits of the hands.

All scans were checked for artefacts. If found to be present, either the participant was rescanned (for example, if a metal clothing clip were detected) after removing the object, or the artefact was excluded from the scan during analysis.

Bone mineral density ($g.cm^2$) is calculated from a DXA scan according to the following:

BMD = BMC / projected area of bone

In the spine, regions of interest (ROI) are usually combined to yield a single measurement from L1-L4 according to the following:

$$BMD_{L1\text{-}L4} = \frac{BMC_{L1}+BMC_{L2}+BMC_{L3}+BMC_{L4}}{Area_{L1}+Area_{L2}+Area_{L3}+Area_{L4}}$$

Measurement of total LM and FM was calculated according to the three-compartment model and android and gynoid mass extracted from data acquired during scanning. Within GE-Healthcare systems the android region extends from the top of the pelvis and terminates at 20% of the total distance between the iliac crests and the neck, the gynoid region includes hips, upper thighs and lower trunk (Imboden et al., 2017).

3.2.7 Magnetic Resonance Imaging (MRI)

Before MRI was undertaken (1.5 T Intera scanner, Philips, The Netherlands), the procedure was explained to the participant, who was asked to complete a screening questionnaire, in the presence of a trained operator, to assess suitability for MRI. Scanning was not undertaken if the participant's answers indicated contraindication(s) to MRI (metal in the body either surgically implanted or from occupational or recreational hazard, intra uterine device not on the MR safety list or sterilisation with metal clips, pacemaker, pregnant, non-removable metal dental work). Before entering the magnet room all metal containing clothing and jewellery and metal in pockets was removed. The scan was performed by a trained operator authorised to perform MRI.

Measurement of areal SAT and VAT was extracted from a single axial slice centred at L3, and volumetric adipose phases from five slices centred around L3, using software present within the scanner. In healthy subjects 97% of the variance in total VAT volume was demonstrated to be explained by a scan image centred at L3 (Demerath et al., 2007), a finding reproduced in a subsequent cross-sectional population study (Irlbeck et al., 2010), Whilst lower accuracy for single-slice estimation of VAT volume has been shown in longitudinal than cross-sectional examination (Shen, Chen, Gantz, Velasquez,

et al., 2012), this approach was preferred as it had the advantage of reducing acquisition time, and thus reducing cost burden, as well as increasing acceptability for participants, compared with scan times for multi-slice and whole-body acquisition, which has been recommended for tracking longer-term changes in AT (Hu, Chen, & Shen, 2016).

3.2.8 Quantitative ultrasound of the calcaneus

In contrast to DXA, which is costly, requires a highly-trained operator, and exposes subjects to ionising radiation, albeit at a low effective dose (1 - 6 microsieverts [μSv]) per scan (Adams, 2013), quantitative ultrasound (QUS) is a readily transportable, rapid and radiation-free method, which can provide information about the structural and material properties of bone (Cavani et al., 2008; Gluer, Wu, Jergas, Goldstein, & Genant, 1994). During bone measurement using QUS, which for studies described in the present document was undertaken at the calcaneus, an ultrasonic soundwave at the frequency of 200 kHz – 1.5 MHz is passed between probes situated either side of the measurement site (Chin & Ima-Nirwana, 2013). Speed of sound (SOS, $m.s^{-1}$), a parameter which reflects ultrasound velocity during transmission through bone, and broadband ultrasound attenuation (BUA, $db.MHz^{-1}$), a measure of signal attenuation which is frequency dependent, are reported by QUS, alongside stiffness index (SI, arbitrary units [AU]), a composite metric derived from SOS and BUA (Hadji et al., 1999) according to the following formula:

$$\text{Stiffness Index (SI [AU])} = (0.67 \times \text{BUA}) + (0.28 \times \text{SOS}) - 420$$

There has been particular interest in QUS as a method of bone assessment in paediatric and young adult populations, in whom effective dose has been shown to be higher for DXA than in adults (Damilakis, Adams, Guglielmi, & Link, 2010). In Study 4, in dancers who trained as usual, and dancers who received supplemental diverse HIIT, mean age was 17 y and 18 y, respectively, and QUS was preferred as a non-ionising method for acquiring calcaneal bone data, as well as for its acceptability and time-efficiency in an applied setting.

In a healthy paediatric population (8 – 13 y), calcaneal SI was moderately correlated with lumbar spine BMC and whole-body BMD in boys (r = 0.30; r = 0.45; $p < 0.01$) and girls (r = 0.32; r = 0.50; $p < 0.01$), respectively, and

in young adult females, SI was found to be better correlated with hip BMD measured by DXA (r = 0.646, p < 0.001) than SOS or BUA independently (Schraders et al., 2019).

CoV for short-term precision for the device used in Studies 2 - 4 was stated as < 2.0% by the manufacturer, and was reported as 2.6: 0.8 – 4.1 (average CoV: range) from 24 studies that have employed the device in calcaneal QUS measurement (Krieg et al., 2008).

Procedure Before a session of scanning, quality assurance (QA) was performed, according to manufacturer instructions.

Separate quantitative ultrasound scans (GE Lunar Achilles Insight, Bedford, UK) were performed on the left and the right heel. The procedure was explained to the participant before the scan was performed. The participant was asked to take off shoes and foot coverings and to sit upright in a supportive chair. The membranes and footwell of the scanner were cleaned using a diluted solution of isopropyl alcohol and this was also applied to the heel to be scanned using a spray. The participant was assisted to place the foot in the footwell, ensuring that the heel was against the edge of the most inferior part of the heel bed and the metatarsal peg was slid down and secured between the first and second metatarsals. The operator checked the sagittal alignment of the participant's lower leg to ensure it was aligned with the longitudinal axis of the foot bed of the scanner and not rotated or deviating along its sagittal axis. The participant was instructed to relax the lower leg against the support which was extended from the back of the scanner. The participant's age and sex were keyed into the scanner using the digital keypad and the scan was initiated. The procedure was repeated for both heels.

A printed report generated by the scanner was used to derive participant values for broadband ultrasound attenuation (BUA, $dB.MHz^{-1}$), speed of sound (SOS, $m.s^{-1}$) and stiffness index (SI, arbitrary units, [AU]). If the printout indicated there had been an error in scanning, the participant was instructed to remove their foot, the bed was inspected and re-sprayed, as was the participant's heel, and the scan procedure repeated. If an error persisted, QA procedures were performed, and the scan repeated once QA was passed.

3.3 Blood collection and analysis

3.3.1 Venipuncture and cannulation

Markers of bone turnover (BTMs) In the non-fasting state, CTX-1, a marker of bone resorption, demonstrates variability according to a circadian rhythm, peaking at ~ 05.00 with a typical nadir at 12.00 (Szulc, Naylor, Hoyle, Eastell, & Leary, 2017). Therefore, blood samples were collected after an overnight fast, which has been demonstrated to attenuate the effect of circadian variability on CTX-1 (Szulc et al., 2017), but has no effect on diurnal cyclicity of P1NP (a marker of bone formation), which was sampled alongside CTX-1 in studies included in this thesis. Moreover, as exposure to exercise is shown to affect bone turnover marker responses in a duration and intensity dependent manner (Maimoun et al., 2006), a gap of 48 hours between latest exercise bout and sample draw was scheduled within blood collection protocols in all training studies.

Procedure To draw serial samples before and after exercise, a peripheral venous access device (BD Venflon Flow 18g [1.30 x 45 mm], Becton Dickinson, NJ, USA) was used to perform cannulation; venipuncture was undertaken using a butterfly needle (BD Vacutainer® Safety-Lok™, Becton Dickinson, NJ, USA).

Insertion and sampling procedures were explained to the participant, who was requested to arrive in a fasted state (water only after 12 midnight). Before the procedure, all equipment was checked to ensure it was in date and then placed on a trolley within access of the couch, which the participant was asked to lie down on. After observing hand washing procedures, the investigator undertaking the procedure put on a clean pair of disposal gloves and palpated the antecubital fossa, as well as visually inspecting the skin, to identify a suitable peripheral vein, where possible aiming to access the non-dominant arm.

The chosen site was prepared for access by wiping with a disposable 70% isopropyl swab and a single use tourniquet applied above the vein to be accessed. After informing the participant that insertion was about to proceed the skin was anchored by applying traction a few centimetres below the access site, to stabilise the vein, and the butterfly needle (venipuncture) or needle and

cannula inserted into the vein at an angle of ~25° with the bevel of the cannula facing upwards. For venipuncture, after flashback of blood was seen the tourniquet was released and the wings of the needle held against the skin, a luer adaptor applied and blood drawn downwards directly into the vacutainer. For cannulation, after the first flashback of blood was seen in the chamber of the needle and blood was visible in the cannula, the cannula was advanced over the needle and into the vein. The needle was withdrawn slightly and after the second flashback of blood along the shaft of the cannula, the cannula was slowly advanced off the needle and into the vein using the dominant hand, whilst the other hand maintained traction to the skin. The tourniquet was released, pressure manually applied to the vein above the cannula tip and the connector applied. The cannula was flushed with saline and adhesive dressings applied to stabilise the cannula position.

To draw a sample, the investigator prepared their hands with isopropyl wipes and put on a clean pair of disposable gloves before removing the white cap from the cannula and wiping the connections with a single use isopropyl swab. After allowing a few seconds for the connections to dry, the cannula was flushed with 1-2 ml of normal (0.9%) saline, previously drawn into a sterile syringe, and ~2 ml of blood drawn and discarded. A luer lock connector was then applied and samples drawn directly into the relevant vacutainer, which was angled downwards to facilitate filling. After completing the draw the adaptor was removed, the cannula was flushed with saline as described, and the white top secured

Samples were drawn in the following order: yellow top (containing serum separator, ~5.0 ml draw to provide serum), green top (containing sodium heparin, ~4.0 ml draw to provide heparinised plasma), grey top (containing sodium fluoride/ potassium oxalate, ~4.0 ml draw to provide whole-blood for glucose and lactate analysis). Each vacutainer was inverted 8 – 10 times to facilitate mixing and placed immediately on ice, apart from yellow top serum separator vacutainers, which were allowed to stand at room temperature for 60 min before being placed on ice.

After the final round of samples were collected, the investigator carefully removed the securing dressings (cannulation only) and applied dry, sterile gauze to the insertion site as the cannula/ butterfly needle was withdrawn,

applying firm pressure to the site for approximately 2 – 3 minutes or until there was no leakage of blood subcutaneously and a suitable dressing was applied to the site. After withdrawal of the cannula it was visibly inspected to check it was complete and undamaged.

At the end of the procedure all materials and sharps were disposed of safely in the appropriate sharps and biohazard disposal bins.

Time and date were recorded, and if an adverse reaction occurred it was written up according to local health and safety procedures; blood spillages were immediately cleaned up using an appropriate sterilising agent. All cleaning materials and dressings were disposed of, in accordance with the local phlebotomy protocol.

3.3.2 Processing of serum and plasma

Vacutainers were centrifuged at 4000 rpm at 4° for 10 minutes. Using a clean pipette for each vacutainer, 2 x 0.5 mL aliquots of serum (gold top tube) 4 x ~0.5 mL aliquots of plasma (EDTA green top tubes) were pipetted into clean eppendorfs labelled with participant ID. Care was taken not to disturb the layers (EDTA) and clot (serum) and if serum appeared haemolysed it was recorded against participant ID.

The resultant eppendorfs of plasma and serum were immediately frozen and stored at -80° until further analysis.

Prior to clinical assay, aliquots were removed from the freezer and transported on dry ice. At the assay destination aliquots were handled and subsequently thawed in accordance with local laboratory procedures.

Description of assay procedure for specific biomarkers is provided within experimental chapters.

3.4 Indicators of exercise intensity

3.4.1 Whole-blood glucose and lactate

An automated device (YSI 2300 STAT Plus Glucose and Lactate Analyzer, YSI, Yellow Springs, OH, USA) was used to measure whole-blood glucose and lactate. Before sampling, daily maintenance was performed on the device, in accordance with manufacturer’s instructions. This included checking fluids (waste, buffer and calibration fluid) and performing QA, by running the

standards as samples to check for linearity against YSI Standards (glucose: 10.0 mmol.L^{-1}; lactate: 5.00 mmol.L^{-1}), with results required to be within 5% of the target. The device was placed in [STANDBY] and activated prior to sampling by keying [RUN] into the device keypad, after the message [READY TO SAMPLE] was displayed [SAMPLE] was keyed in and a vacutainer (grey top, fluoride) with well-mixed whole-blood introduced beneath the sipper. During the draw, the vacutainer was held still to avoid contact with the sipper. After sampling was completed (~4 s) and the sipper had withdrawn automatically, the vacutainer was placed in an appropriate hazard disposal bin. Results were read off from the automated print out and recorded against participant ID. The sipper was cleaned regularly, according to manufacturer's recommendation, using a 70% isopropyl swab.

Precision for YSI (2300) cited by the manufacturer is ±2% of the reading or 0.2 mmol.L^{-1} for glucose and ±2% or 0.1 mmol.L^{-1} for lactate, depending on whichever of the two figures is larger. CoV for whole blood (WB) glucose detection has been demonstrated at <2% (Miller, 2017) and 0.8 – 3.3% for WB lactate (Foxdal, Bergqvist, Eckerbom, & Sandhagen, 1992).

3.4.2 Capillary blood sample

In an applied setting, a portable device (Lactate Pro 2™, Arkray, Shiga, Japan) was used to measure lactate from a freshly drawn blood sample.

The device was acclimatised to ambient conditions for ~20 minutes before data gathering was initiated and the procedure was explained to the participant. Before each sample, an unused, in date strip (Lactate Pro 2™ test strip) was opened with clean, dry hands and introduced into the analyser until a beep was heard and the current date and time appeared, indicating the device was ready to sample. To take a sample from a participant a digit was selected and wiped with a single use isopropyl swab, after waiting a few seconds for the alcohol to dry the skin on the finger pad was punctured using a disposable, single use lancette (Accu-Chek Safe-T-Pro Plus Disposable Lancet, Hoffman-La Roche, Basel, Switzerland) and the surrounding skin gently squeezed until a drop of blood formed. This was wiped away and the skin gently squeezed to form another drop of blood, and the test strip immediately introduced at an angle of 90°. After the window on the strip indicated a full draw (0.3µL), the result displayed after the 15 s countdown was recorded against participant ID,

according to the schedule of sampling. After recording the result, the test strip was removed and securely disposed off in an appropriate hazard bag.

If an unexpected value was returned, the device was checked to ensure the thermometer symbol was not on, indicating the device was not within manufacturer's recommended operating range for temperature and humidity, and a fresh test strip used to perform a further test, ensuring the strip was filled with enough blood to return a result.

Against a criterion standard (Radiometer ABL90, Radiometer Medical ApS, Brønshøj, Denmark), CoV for Lactate Pro 2^{TM} has been shown to be 0.0% for the lactate concentration band 0 - 4.9 mmol.L^{-1}, 1.0% for 5.0 – 9.9 mmol.L^{-1} and 0.0% for >10 mmol.L^{-1} , with corresponding bias for these bands against the criterion of -0.06%, -1.48% and -1.81%, respectively (Bonaventura et al., 2015).

3.5 Locomotor profile

Global positioning system (GPS) tracking has been incorporated into wearable technology and is now widely used in professional and non-professional sport to monitor performance and provide activity-related metrics, such as speed, locomotor distance and accelerations. Motion trackers use GPS data from orbiting satellites, originally developed for military use, to calculate the unit wearer's position according to changes in satellite signal frequency as a result of locomotor activities (Larsson, 2003). A small unit is typically positioned between the shoulder blades, to maximise exposure to the satellite signal, in a compression vest worn close against the body to minimise movement artefact. Investigation of an early wearable system (GPS 45, Garmin, Garmin Ltd., Kansas, USA) reported error of ~2 m and precision of 0.6 m for distance (Schutz & Herren, 2000), however subsequent iterations of the technology have demonstrated greater validity for higher (5 Hz) versus lower (1 Hz) sampling rates (Aughey, 2011).

As velocity of motion increases, reliability of measurement for GPS has been found to decrease (Petersen, Pyne, Portus, & Dawson, 2009), leading to concerns about accuracy of the technology for quantification of high-demand activities (Cummins, Orr, O'Connor, & West, 2013). Despite these limitations, GPS has been used to characterise physiological responses to training load

(Buchheit, Mendez-Villanueva, Simpson, & Bourdon, 2010), and triaxial acceleration data has been shown to provide robust metrics for intermittent team sport activities and multidirectional locomotor actions (Barrett et al., 2016).

Recently, GPS technology has been partnered with inertial sensors to enable temporal, kinematic and dynamic parameters to be estimated with greater precision (Camomilla, Bergamini, Fantozzi, & Vannozzi, 2018), using miniaturised microelectromechanical systems (MEMS) technology to provide angular and linear acceleration data according to three orthogonally mounted sensing axes. High levels of agreement between MEMS and force plate data have been demonstrated in the vertical direction during characterisation of vertical jumping (Setuain et al., 2016), and the technology has been proposed to be adequate, in an applied context, for capacity assessment in the vertical direction (Camomilla et al., 2018).

Typically, in team sport application, data recorded by the motion tracking unit is exported to a cloud based system and, depending on metrics specified by the user, a report generated documenting time spent and distance covered in threshold-derived locomotor categories (e.g. walking, running, high speed running, sprinting), using software specific to the device manufacturer.

In Study 1, during outdoor football participation, GPS units sampling at a frequency of 5 Hz were assigned to participants during SSG, and locomotor profile characterised from manufacturer-derived outputs. In a study that examined application of GPS to profile linear football actions CoV was 4.6 – 5.3% for 5 Hz (Portas, Harley, Barnes, & Rush, 2010), and 7 – 14% for accelerations at this sampling frequency against a criterion of a laser (Aughey, 2011). Therefore, whilst participants were tested and re-tested using the same unit, under standardised conditions for acquisition of data, as recommended to reduce measurement error for this device (Jennings, Cormack, Coutts, Boyd, & Aughey, 2010b), high speed and changes of direction (COD) are unlikely to have reflected ‘true’ values, due to limitations inherent in the technology demonstrated for 5 Hz GPS (Jennings, Cormack, Coutts, Boyd, & Aughey, 2010a)

In Studies 2, 3 and 4, a MEMS unit, providing sampling at a frequency of 100 Hz, was worn during exercise, and raw data were exported from the unit to

characterise locomotor profile according to durations of triaxial accelerations, as described below. To the author's knowledge, this has not been attempted previously either in an elite indoor athlete population, as undertaken in dancers in Studies 2 and 4, or for an intervention replicating team sport actions in the format of pre-determined multidirectional choreography, as in Study 3. Unusually, the aim was not to provide activity classification, which may be preferable in a team sports environment, but to represent directional and threshold characteristics of the acceleration signal in relation to investigative outcomes, specifically potential osteogenic effects and physiological demands for a bout of diverse exercise. It was therefore not possible to compare the present approach to treatment of accelerations against an ecologically validated criterion, as has been recommended (Camomilla et al., 2018). In any case, algorithms developed within specific sports have demonstrated lack of transferability across activity codes, which may be attributable to differences in peak acceleration profile demonstrated during code-specific high-demand actions (Wundersitz, Gastin, Robertson, Davey, & Netto, 2015). This has been examined in athlete populations by comparing data from from wearable technology against a validated local positioning system, as undertaken for soccer (Stevens et al., 2014) and cricket and tennis (Vickery et al., 2014), however the focus has been on accuracy in representation of distance covered and speed during progressive actions, which are critical determinants of workload in field-based athlete environments, and studies in recreational and dance populations are not represented. However, comparison of raw cranio-caudal (i.e. axial) accelerations from a trunk-worn triaxial MEMS device, which was the same brand and iteration as the device used in studies described here, has shown the method to be valid for estimating vertical forces during running and COD tasks, when compared against force plate data (Wundersitz, Netto, Aisbett, & Gastin, 2013). Evidence of good validity in the vertical direction, against a criterion standard, for the method used to quantify locomotor characteristics in Studies 2 – 4 offers support for the investigative approach adopted, which focussed on vertical acceleration profile as a critical determinant in the relationship between locomotor characteristics and osteogenic effects of diverse exercise.

There is some evidence in the literature for reliability of derivatives of the acceleration signal in characterising exercise demand. For example, whilst

acceleration derived from MEMS has demonstrated low correlation with mechanical load in a team sport environment (Nedergaard et al., 2017), acceptable within- and between- device reliability has been shown for rate of change of acceleration characterised by jerk amplitude (Boyd, Ball, & Aughey, 2011) and near perfect (r = 0.92 - 0.98) correlation within an individual athlete with VO_2 and heart rate as indicators of exercise intensity (Barrett, Midgley, & Lovell, 2014). These studies tend to support the approach adopted in order to profile locomotor actions during acute and chronic implementation of diverse exercise in the novel format devised.

For linear and acceleration data, bands were created (linear: g bands; angular R bands) in order to quantify time spent within thresholds, to indicate the osteogenic potential of the movement patterns.

3.5.1 Procedure

Locomotor profile was measured using a wearable motion tracking device. The motion tracking unit was secured in custom fit pockets stitched into close-fitting training vests supplied by the manufacturer, care was taken to ensure a standardised alignment of the device, which was aligned axially, between the scapulae. If participants were of lean and small stature, an extra vest was worn to ensure movement artefact was minimised.

Where logistically possible, each participant was assigned a unit, which was used on each occasion of data acquisition.

To acquire exercise heart rate (HR) data, each participant wore a belt with an integrated HR monitor (Polar T31, Polar, Finland) which synchronised with the motion tracking unit during exercise. The electrode region of the HR unit was dampened prior to skin contact and the belt was secured closely against the skin, at the level of the xiphoid process, by adjusting the HR belt. The motion tracking unit was activated prior to exercise and the participant stood quietly for 10 s maintaining an upright posture, to ensure a stable signal was established (GPS) and to provide controlled conditions for initiation of the locomotor signal (indoor acquisition of accelerometry profile). This procedure facilitated zeroing of motion sensor data during post-acquisitional parsing and analysis. The unit was de-activated immediately once exercise was completed, and time, date and unit ID noted against participant ID.

3.5.2 GPS data download and processing

After exercise, the unit was placed in the download cradle supplied by the manufacturer and session data were downloaded to a personal computer.

Customised software (SPI IQ; formerly Team AMS. GPSports, Canberra, Australia) was used to extract raw data from the unit download and a report generated, summarising distance covered according to pre-determined locomotor categories (walking, running, high speed running, sprinting) and counts of accelerations and decelerations, according to three zones (0.6 - 1.0 $m.s^{-2}$, 1 - 1.4 $m.s^{-2}$, >1.4 $m.s^{-2}$).

Heart rate data were also extracted from the download and reported as mean, maximum and minimum heart rate during exercise.

3.5.3 Accelerometry profile of indoor locomotor activity

Triaxial linear triplanar angular acceleration data, acquired by the MEMS-enabled motion tracking unit worn during exercise, were downloaded automatically when the unit was docked. Raw accelerometry data in three orthogonals (forwards, sideways and up) and three planes (roll, pitch and yaw) were exported as a .csv file for further analysis.

A convention was adopted of reporting data as ‘accelerations’ as the device was not enabled to discriminate between a linear acceleration and a negative acceleration (i.e. acceleration in the opposite direction). Linear data were reported as accelerations ‘forwards’ to denote forwards-backwards, ‘sideways’ to denote left-right, and ‘up’ to denote upwards-downwards, respectively, without discrimination of decelerations from accelerations.

Angular acceleration profile data were exported as individual data sets designated as ‘roll’, ‘pitch’ and ‘yaw’ (manufacturer’s description). It was established, by wearing the unit to perform a standardised series of trunk motions, that the planar trunk movements these data sets described corresponded to angular accelerations in the axial, sagittal and frontal planes, respectively. In locomotor terms these were reported as left-right trunk rotation, trunk forward-backward bending (spinal flexion-extension) and trunk sidebending to the left and right.

Heart rate data were also extracted from downloaded MEMS data and an excel script used to provide mean, maximum and minimum exercise heart rate.

3.5.4 Analysis of linear and angular acceleration data

Linear acceleration data corresponding to accelerations forwards, sideways and up, and angular acceleration corresponding to roll (axial rotation), pitch (trunk forwards-backwards bending) and yaw (trunk sidebending), were treated as separate data sets. Data for each variable were zeroed at baseline, and absolute values used in analysis. After subsequent inspection of the data, bands were established according to the aim of preserving signal discrimination (i.e. having sufficient numbers of bands to illustrate the range of data values), whilst avoiding reduction of statistical power by having too many bands with small amounts of data contained within them (i.e. having sufficient data present within each band). Therefore, even though a putative osteogenic threshold of 3.6 g has been suggested (Jämsä, Vainionpää, Korpelainen, Vihriälä, & Leppäluoto, 2006), thresholds were established relative to data provided by participants, rather than by calibration against external data presented in the literature, such as there is, that has adopted a similar approach.

Given the sampling rate of 100 Hz, duration spent within each acceleration band was calculated by multiplying counts within each epoch according to the following:

Duration (s) = (Counts within epoch) x 0.01

For acceleration data extracted from submaximal exercise, the highest three acceleration bands were collapsed to a single upper band, designated ‘above 2 g’ (linear acceleration data) and ‘above 90 degrees.s^{2}’ (angular acceleration data). This decision was taken after inspection of raw data, and according to the hypothesis that accelerations in the upper three epochs correspond to higher intensity performance and constitute the anabolic derivative of the acceleration signal.

3.6 Perception of effort during exercise

Rating of perceived exertion (RPE) is a simple and inexpensive method for assessing subjective evaluation of physiological demands experienced during a bout of exercise. RPE has been positively correlated with exercise

heart rate (r^2 = 0.62), blood lactate (r^2 = 0.57) and oxygen uptake ($\dot{V}O_2$) (r^2 =0.61) (Chen, Fan, & Moe, 2002) and, as an approach to estimating exercise load, has the advantage of resolving a psychological aspect of effort perception (Morgan, 1994). The most commonly used RPE scale, the 10-point Category Ratio scale (CR-10) (Borg & Kaijser, 2006a), utilises a 1 – 10 scale, with verbal indicators used to anchor specific numbers in the scale relative to intensity.

Alternatively, a questionnaire using a visual analogue scale (VAS) may be used to assess effort by providing a continuous outcome variable, which is calculated from distance marked along a horizontal line anchored at either end by an indicator of 'lowest' to 'highest' severity. This approach has shown good agreement with the Borg scale, for example in assessment of effort during an incremental arm crank task (Capodaglio, 2001), and has been used to assess subjective responses in a recreational team sport intervention (Bangsbo et al., 2010) and to monitor playing load in elite footballers (Rebelo et al., 2012). Furthermore, a differential version of the VAS scale has been used to dissect perception of global exercise response in an intervention which compared responses to incremental running and pedalling using this approach (Ueda, Nabetani, & Teramoto, 2006). This suggests it may be possible to examine how dimensions of the exercise signal are perceived categorically, for example demand on lower limbs versus demand on breathing, and may provide greater discrimination in comparison of acute and longitudinal perceptual responses to exercise.

3.6.1 RPE

The participant was provided with a training diary which contained a printed scale for assessment of perceived exertion (Borg & Kaijser, 2006b). After each home exercise session the participant was asked to record their answer to the question: 'How hard was today's training?' by choosing the number for the description that corresponded most closely to their perceived response, on a scale of 1: 'really easy', to 10: 'maximal'. This scale was used after supervised exercise testing under controlled conditions.

3.6.2 VAS

Immediately after training the participant was asked to rate perception of exercise according to three categories: overall physical challenge, challenge on

the legs and challenge on breathing. To indicate responses the participant placed a mark along a horizontal line, representing a visual scale extending from left ('No exertion at all') to right ('Maximal Exertion'), placed beneath each category of challenge. A score for each response category ($VAS_{CHALLENGE}$, VAS_{LEGS}, $VAS_{BREATHING}$) was calculated by measuring the distance of the mark (cm) from the left-hand edge of the score line and dividing by the total length of the line. A single value (VAS_{TOTAL}), representing overall response to the session, was calculated by adding together the scores from each category.

3.7 Submaximal Exercise

Whereas submaximal exercise provides an approach to predicting maximal cardiorespiratory fitness in patient or elderly populations, who may be probhibited from maximal testing due to frailty or comorbidities (Noonan & Dean, 2000), within regular athlete monitoring, repeatable, lower intensity submaximal tasks enable adaptations to training to be evaluated, without the necessity for time loss and recovery after maximal/ exhaustive testing (Lamberts, Swart, Noakes, & Lambert, 2011). In alignment with the latter approach, in Studies 1 - 4 submaximal exercise was implemented, with the aim of comparing performance during a standardised locomotor task, either between interventional groups under baseline conditions (acute testing), or after chronic administration of an exercise intervention, in order to estimate training response in HR, blood lactate and accelerations derived from quantification of locomotor profile.

Procedure To complete the submaximal exercise challenge, the participant jogged forward for 6 s, keeping pace with instructions from a pre-recorded soundtrack (Yo-Yo Intermittent Endurance Test Level 1 [YYIE1], levels 1 - 5) (Bradley et al., 2011), and turned 180 degrees around a cone placed 12 m from the start line. After completing three 12 m sagittal runs at this pace, the participant was instructed to turn 90 degrees and traverse the 12 m distance between the cones twice, performing progressive lateral steps, at a pace of 9 s for each shuttle. Two further repetitions of this five shuttle sequence were repeated, at a pace of 5 s for each sagittal and 8 s for each frontal shuttle (ramp 1), ramped to 4 s (sagittal) and 7 s (frontal) for completion of the final sequence of progressive shuttles.

Data analysis Standardised trace lengths (120 s) were used to quantify heart rate parameters for all participants who provided submaximal data.

3.8 Measurement of balance

The Flamingo Balance Test, which forms part of the Eurofit test battery for assessment of physical fitness (Adam, Klissouras, & Ravasollo, 1988) was used to assess balance. The test has been positively correlated (r = 0.64; r = 0.56, right and left leg, respectively) with the Stork Test, another assessment used to evaluate balance (Kranti Panta, 2015), however some researchers have questioned Flamingo Balance test reliability after large variation was observed, in a study in undergraduate students into test-retest reliability of the Eurofit battery (Tsigilis, Douda, & Tokmakidis, 2002). It was decided to use the Flamingo Balance to enable results to be considered alongside those from other interventions (Helge et al., 2010; Krustrup, Hansen, et al., 2010), which have used this test as an outcome measure to assess the effects of recreational football prescription.

Procedure Prior to measuring balance using the Flamingo balance test, the participant was familiarised with the unilateral balance task and given two opportunities to practise their preferred stance. The test required 60 s of single leg balancing on the non-dominant leg on a raised wooden platform (2.5 cm wide x 60 cm long) 6 cm off the ground. The timer was stopped when a participant stepped off the platform and restarted as soon as unilateral stance was re-established. After 60 s balancing was completed, the test score (counts) was the number of episodes of loss of balance requiring the participant to step off the platform.

4 Effects of 16 weeks Small-Sided Football or Whole-Body Vibration on Bone - A Randomised Controlled Intervention with Brief Diverse Exercise in Sedentary Premenopausal Females

4.1 Introduction

Regular physical activity (PA) can mitigate and even prevent deleterious health outcomes from an array of chronic diseases (Hojman, Gehl, Christensen, & Pedersen, 2018; Pedersen, 2019; Rhodes, Janssen, Bredin, Warburton, & Bauman, 2017). Whilst transcriptional and epigenetic processes underlying adaptations to exercise have been described (McGee & Hargreaves, 2020; Neufer et al., 2015), the relationship between training dose and performance intensity provides an amenable mechanism for leveraging effects of exercise in an applied setting (Ruegsegger & Booth, 2018). Furthermore, according to the proposal of 'exercise as medicine' (Pedersen & Saltin, 2015), identifying the effectors of musculoskeletal (MSK) responses to PA, and administering them deliberately, in an acceptable format, may provide an approach to targeted exercise prescription.

As a form of codefied activity, team sports have features in common with high-intensity intensity interval training (HIIT), which has been examined in healthy and patient populations (Gibala, Little, MacDonald, & Hawley, 2012) and found to be a more time-efficient mode of delivering exercise, matched for energy expenditure, compared with moderate-intensity continuous training (MICT) (Weston et al., 2014). Activity in HIIT, and typically in team sports, is intermittent and characterised by intense phases of whole-body actions, which elicit high heart rate, followed by fallow periods of recovery. Analysis of locomotor characteristics during football participation has confirmed a profile of intermittent activity in elite (Harper, Carling, & Kiely, 2019) and general populations (Randers et al., 2010). However unlike HIIT, where blocks of 60 – 240 s of strenuous activity at > 80% of maximum heart rate (HR_{MAX}) are prescribed (Keating, Johnson, Mielke, & Coombes, 2017), studies that have implemented 'football for health' have tended to deploy 60 min sessions 2 – 3 times per week (Bangsbo, Junge, Dvorak, & Krustrup, 2014; Krustrup, Hansen, et al., 2010). Therefore, the effect of reducing bout duration on MSK and

cardiometabolic health outcomes has not been demonstrated for recreational football in a general population.

Although implementing bouts of low duration is not represented in football interventions in general populations, reducing pitch size and player numbers to elicit a more intense exercise stimulus has been investigated in both elite (Hill-Haas, Dawson, Impellizzeri, & Coutts, 2011) and non-elite populations (Zouhal et al., 2020). Evidence of beneficial effects of training with small-sided games (SSG) has focussed on ecologically relevant adaptations in team sports athletes, notably greater capacity to cover distance at higher speed (Halouani, Chtourou, Gabbett, Chaouachi, & Chamari, 2014), and perform consecutive bouts of sprinting and change of direction (COD) (Bujalance-Moreno, Latorre-Román, & García-Pinillos, 2019). Whereas physiological adaptations that enhance intermittent high-intensity performance, a characteristic of team sport demands (Bangsbo, 2015), are important in athletes, advocacy for SSG in a recreational setting has cited broad-spectrum benefits to cardiovascular, metabolic and musculoskeletal health (Krustrup, Hansen, et al., 2010), alongside positive effects on bone (Milanović et al., 2019).

Analysis of locomotor characteristics in semi-elite players has shown that workload, an algorithmically derived metric that includes accelerations and sprinting at higher thresholds (Theodoropoulos, Bettle, & Kosy, 2020), increases under intensive conditions (i.e. SSG) compared with extensive conditions (i.e. 11 vs 11 on a full pitch) (Casamichana, Castellano, & Castagna, 2012; Rebelo, Silva, Rago, Barreira, & Krustrup, 2016), with a higher percentage of bout duration spent at 85 – 90% HR_{MAX} alongside elevated blood lactate post-SSG (Aguiar, Botelho, Lago, Maças, & Sampaio, 2012). The increase in workload may be attributable to more frequent interactions between players during SSG, as a result of reduced pitch dimensions, but may also reflect an increase in absolute participation, as positional play is effectively obliterated under the condition of reduced player numbers. Therefore, according to the proposal that characteristic football actions elicit cardiometabolic and MSK adaptations reported for interventions in a recreational setting (Bangsbo et al., 2010; Krustrup, Hansen, et al., 2010), it may be hypothesised that SSG provides a superior adaptogenic stimulus than football in more extensive formats, as a result of the increase in workload imposed by SSG conditions.

Investigators who have conducted football training studies have attributed evidence of anabolic effects of participation to high magnitude and diverse skeletal strains elicited by multidirectional participatory activities (Helge et al., 2010; Krustrup, Helge, et al., 2018; Ubago-Guisado et al., 2015). This interpretation is in agreement with evidence that exercise-derived mechanical strains exceeding those prevailing in habitual activity can influence bone metabolism at a systemic level, and/or induce localised adaptation in a site-specific manner (Lombardi, 2019; Ozcivici et al., 2010; Thompson, Rubin, & Rubin, 2012). Therefore, according to the analogy of 'football as medicine' (Sarmento et al., 2020), it follows that bone responses to SSG could be enhanced, compared with football in an extensive format, as a result of amplifying the 'dose' of actions proposed to constitute the bone-anabolic derivative of biological signals generated by exercise (Rubin & Lanyon, 1985).

Although there is evidence for interventional SSG supporting both systemic and regional effects on bone (Krustrup, Helge, et al., 2018), the osteogenic effects of SSG in brief prescription have not been represented in the literature. For example, in a 16 week football training intervention in premenopausal women (29.8 ±5.8 y) whilst no changes were seen in total or regional BMD, background plasma osteocalcin (OC), a biomarker of bone formation, was increased by 37%, at a dose of 2 x 60 min SSG per week, (Jackman et al., 2013). However, in a 15 week randomised controlled intervention (RCT) that compared 3 x 60 min SSG with high intensity and endurance swimming in sedentary middle-aged (45.6 y) women, whilst total BMD did not change in any group, femoral BMD increased by 2.4 ±2.9% and BMD at the greater trochanter by 1.7 ±1.9%, accompanied by significant elevations in background OC, N-terminal propeptide of Type-1 procollagen (P1NP, marker of formation) and C-terminal telopeptide of Type-1 collagen (marker of bone resorption), but only in the group assigned to SSG (Mohr et al., 2015). Comparing results from Mohr and colleagues with those reported by Jackman et al, it could be hypothesised that increasing the volume of weekly exposure to SSG may have contributed to regional bone adaptation reported in the former study, in response to a higher cumulative dose of exposure to the stimulus provided by SSG. However, whilst effects of longer (60 min) bouts of SSG on background bone biomarker status have been previously examined in recreational populations (Krustrup, Williams, et al., 2018), acute responses to

brief SSG exposure have not been described, nor how these responses vary after chronic SSG at reduced bout duration. According to evidence of structural adaptations in trabecular bone, attributed to transient elevations in parathyroid hormone (PTH) associated with brief bouts of running (Gardinier, Mohamed, & Kohn, 2015), it may be hypothesised that transient, as well as constitutive, increases in biomarkers of bone metabolism could explain anabolic effects on bone reported for SSG. Therefore, characterising bone biomarker profile immediately after exposure to brief SSG, as well as at rest, could provide novel insight into potential quantal phenomena of acute dose-response effects of exercise on bone metabolism.

In contrast to the locomotor environment of football, which is characterised by frequent, autonomously regulated changes in activity in both recreational (Randers et al., 2010) and elite settings (Rampinini, Coutts, Castagna, Sassi, & Impellizzeri, 2007), whole-body vibration (WBV) is a passive mode of activity that excludes impact, and elicits mechanically induced strains via reflex mediated tendon-muscle interactions in response to platform oscillations (Rittweger, 2010). RCTs have examined the potential of WBV as an acceptable exercise modality, particularly in frail populations, such as the elderly, for whom impact exercise may be prohibited, however evidence of an osteogenic effect for WBV is controversial. For example, a meta-analysis of studies conducted in older females, younger adults and paediatric populations concluded that whilst there is support for WBV increasing BMD in postmenopausal females at the hip, and in children in the spine and tibia, an osteogenic effect of WBV in young adults was not supported (Slatkovska et al., 2010). Although differences in administration and lack of consistent methodology across protocols may explain some of the variation in responses, as highlighted in critical appraisal of investigations into WBV (Rauch et al., 2010), it is unknown whether brief bouts of WBV provide a stimulus exceeding habitual background PA and therefore could elicit an osteogenic response in a healthy, premenopausal female population.

Although, as has been suggested, conflicting results for WBV may be attributable to differences between vibration protocols (Marin-Cascales et al., 2018), it has recently been shown that transmission of accelerations to the spine is enhanced at low frequency and high amplitude (i.e. larger platform

displacement) (Zaidell et al., 2019), which suggests there may be conditions of optimum WBV prescription for bone adaptation. Therefore, administering WBV as a progressive protocol of brief, low frequency, high amplitude exposures, in alignment with the findings of Zaidell and colleagues, with the aim of amplifying osteogenic effects of exercise, has the potential to elucidate whether WBV provides a sufficient stimulus to bone adaptation in premenopausal females. Furthermore, compared with SSG, WBV provides a distinct mechanical strain environment, particularly for vertical accelerations, which arise passively during WBV as a result of platform oscillations, whereas in SSG vertical accelerations would be associated with ground reactions forces (GRFs), generated by foot strikes during sprinting, jumping and COD (Randers et al., 2010). Comparing responses to accelerations encountered within distinct mechanical strain environments, by matching exposure to either SSG or WBV, could elucidate whether mode of application of vertical accelerations is a determinant of osteogenic outcome in premenopausal females. Moreover, as few studies have undertaken assessment of acute bone biomarkers before and after brief exercise, quantifying bone biomarker profile in the untrained and trained state, to a bout of either WBV or SSG, would provide novel insight into bone metabolic responses to brief exercise that either excludes, or includes, vertical impacts and GRFs.

In summary, this controlled study aimed to quantify the effects of 16 weeks training with twice weekly, low duration (13.5 minute) bouts of either SSG or WBV on bone, by sampling plasma biomarkers of bone metabolism pre- and post-exercise and using DXA to measure body composition, to quantify osteogenic effects for low-dose exercise, in distinct strain environments, in premenopausal females.

4.2 Methods

4.2.1 Study design

This was a randomised controlled training intervention, designed as a pilot to investigate the effects of brief exercise on bone, using whole-body vibration (WBV) and small-sided football training (SSFT) in sedentary premenopausal women. Bone mineral density (BMD) and lean and adipose tissue mass in regions of interest (ROI) were assessed by dual x-ray absorptiometry (DXA) and compared pre- and post- 16 weeks between

interventional groups. Biomarkers of bone metabolism were examined in participants allocated to either WBV or SSFT, due to limitations on resources. A power calculation to establish participant numbers for each arm of the intervention was not conducted as effect size data were not available, due to the novel dimension of prescribing SSFT at low bout duration. After considering evidence from previous studies, which have examined the effects of SSFT using longer duration bouts, it was aimed to recruit 25 participants to each training arm of the study and a further 20 participants to account for drop-out and non-compliance (95 participants in total). It was subsequently calculated that at n = 25 participants per arm, the study would be sufficiently powered to detect 1% change in hip BMD measured by DXA.

Data collection from all participants was undertaken at baseline and repeated after 16 weeks. In participants randomised to training arms of the study (WBV or SSFT) additional data were collected to measure physiological responses to the acute exercise stimulus. In WBV, heart rate was recorded during training sessions; in SSFT, heart rate, effort perception and movement characteristics were recorded during early- (1 - 4 weeks), mid- (7 - 10 weeks), and late- (13 -16 weeks) phases of the intervention, as described below in 4.2.12.

4.2.2 Eligibility, recruitment, randomisation

A total of 43 women participated in the study (mean age 39.1 ± 4.8 years; range: 28 to 46 years [including those aged 45 at entry]), which recruited via poster campaign in community venues, newspaper advertisements and announcements in the local press and radio stations.

A pre-screening questionnaire was administered by telephone, to assess suitability for entry onto the study according to the following criteria:

Inclusion criteria:

- Female
- Premenopausal
- Menstruating regularly
- Sedentary (defined as < 150 min weekly participation in activities likely to increase cardiac output)

- Aged 25 – 45 y

Exclusion criteria:

- Pregnant or attempting to become pregnant
- Taking medication known to affect bone metabolism
- Fracture or episode of lower back pain in the previous six months
- Current musculoskeletal issue preventing participation in WBV or SSFT

After acceptance onto the study, participants were randomly assigned to one of three groups: control (CON, *n* = 14), low-magnitude WBV (VIB, *n* = 16) and SSFT (FG, *n* = 13), using a random number generator programme, with numbers previously assigned to each study arm (e.g. WBV: 1 – 25, etc.). Participants were instructed to continue habitual PA, which was supplemented in VIB and FG by twice weekly sessions of either 13.5 min WBV (VIB) or SSFT (FG).

Written informed consent was obtained from all participants, after they had been informed verbally and in writing of the benefits, risks and possible discomforts associated with taking part in the study, and had had all procedures explained to them by an investigator. The study was approved by the National Research Ethics Service (NRES) (12/SW/0045) and the University of Exeter research ethics committee (NHS 2012/329).

4.2.3 Activity and dietary status

To assess physical activity (PA) status and gather information about lifestyle, dietary habits and medical history, participants completed a bone questionnaire on entry to the study (see Appendix A1.1 for a blank version of the questionnaire).

A food frequency questionnaire (CaQ version 2007, National Institutes of Health, Bethesda, MD 20892-1078), validated for assessment of weekly calcium intake (Sebring et al., 2007), was completed at baseline (see Appendix A1.2). Participants were asked not to change their dietary patterns, or restrict food intake, during the intervention period.

Tobacco and alcohol consumption, contraceptive use and daily calcium intake are summarised, alongside baseline characteristics, are summarised in Table 1 for participants who completed the intervention.

Table 1 Baseline Characteristics (Mean ±SD)

	Intervention Group		
Characteristic	**CON (*n* = 14)**	**SSFT (*n* = 13)**	**WBV (*n* = 16)**
Age (y)	40 ±5	39 ±6	39 ±4
Height (m)	1.66 ±0.07	1.65 ±0.05	1.69 ±0.05
Weight (kg)	68.6 ±13.9	67.9 ±10.2	72.2 ±13.5
BMI ($kg.m^2$)	24.9 ±4.9	24.9 ±3.8	25.2 ±4.5
HR (bpm)	80 ±10	77 ±7	74 ±11
Systolic (mmHg)	117 ±14	120 ±17	123 ±13
Diastolic (mmHg)	75 ±9	77 ±11	77 ±9
MAP (mmHg)	89 ±10	91 ±13	92 ±9
Ca ($mg.day^{-1}$)	1266 ±539	1642 ±991	1416 ±485
Caffeine (daily)	1-5	1-5	1-5
Alcohol (weekly)	6-10	6-10	6-10
CU (%)			
- OCU	15	8	13
- Coil	23	17	6
- N	62	75	81

Note. Continuous data are displayed as mean (±SD); categorical data as range (weekly consumption) or median (Contraceptive Use [CU]).
CON: Control; SSFT: Small-Sided Football Training; WBV: Whole-Body Vibration Training; BMI: Body Mass Index; MAP: Mean Arterial Pressure; Ca: Calcium daily intake; Caffeine: response to 'cups of caffeinated beverages per day'; Alcohol: response to 'units of alcohol per week'; CU: Contraceptive Use; OCU: Oral Contraceptive Use, N: answering 'No' to: 'currently using contraception (pill, coil or implant) Yes or No'.

4.2.4 Anthropometric data collection

Participants were asked to remove footwear and measurement of body mass (kg) (see section 3.2.1) and height (m) (see section 3.2.2) was performed.

4.2.5 Heart rate and blood pressure at rest

Participants were instructed to sit quietly for ten minutes before heart rate and blood pressure were measured at rest, and estimation of mean arterial pressure calculated (see General Methods section 3.2.5).

4.2.6 Procedure and schedule for blood samples

Participants for WBV and SSFT were familiarised with procedures for blood collection before baseline data collection. They were instructed not to take part in PA other than their usual activities for 48 h and to arrive fasted (water consumption only after midnight). Between 8 and 8:30 a.m and before undertaking undertaking exercise, a cannula was inserted the first sample was collected (see section 3.3.1). A further sample was collected immediately after exercise, 30 min after exercise, and 48 h after exercise (fasted state). Samples for analysis of biomarkers of bone turnover were placed on ice before being processed and stored at - 80° C, as described in section 3.3.2.

Blood sampling was repeated at the final training session, under the same conditions, and according to the same schedule (pre-, immediately after, 30 min and 48 h after exercise), after 16 weeks participation in either SSFT or WBV.

Lactate and glucose

Whole venous blood was collected into NaFluoride tubes, according to the schedule of sampling described, and stored on ice to avoid degradation, before being analysed (see section 3.4.1).

Biomarkers of bone turnover

Three bone turnover markers were measured using chemiluminescent immunoassays. For bone resorption, plasma C-terminal telopeptide of Type-1 collagen (pCTX-1) was assayed (IDS-iSYS CTX-1 (CrossLaps ®) (Immunodiagnostic Systems PLC, Tyne and Wear, UK), and for bone formation, plasma N-terminal propeptide of Type-1 procollagen (pP1NP) and plasma osteocalcin (pOC) were assayed using the IDS-iSYS intact P1NP and N-Mid Osteocalcin assays (Immunodiagnostic Systems, Tyne & Wear, UK).

All three assays were performed on a dedicated iSYS automated analyser in accordance with the manufacturer's instructions, and in a laboratory conforming to the International Standard specifying the requirements for quality and competence in medical laboratories (DS/ EN ISO 15189:2013).

The following procedure was observed for all three assays: plasma aliquots were kept frozen at -80 degrees until the day of analysis; samples were analysed using a single batch of the relevant immunoassay, and for each

analysis the manufacturer's control specimens were used to verify assay performance.

Expressed as a coefficient of variation, the intermediary precision for CTX-1 was 5.3%; 3.4% and 3.5% at pCTX-1 concentration 0.0213 $\mu g.L^{-1}$; 0.869 $\mu g.L^{-1}$ and 2.113 $\mu g.L^{-1}$, respectively. For pP1NP the intermediary precision was 5.4% (18.96 $\mu g.L^{-1}$); 6.5% (48.68 $\mu g.L^{-1}$) and 6.1% (122.10 $\mu g.L^{-1}$), and for pOC 3.0% (8.73 $\mu g.L^{-1}$); 3.6% (27.58 $\mu g.L^{-1}$) and 3.5% (68.70 $\mu g.L^{-1}$). The analyte-specific lower limit of quantification according to manufacturer derived values was 0.03 $\mu g.L^{-1}$ for pCTX-1; 2.0 $\mu g.L^{-1}$ for pP1NP and 2.0 $\mu g.L^{-1}$ for pOC.

4.2.7 Exercise protocol

SSFT Participants trained twice per week for 13.5 min with sessions arranged to allow 48 h between training bouts; a minimum gap of at least 24 h between sessions was permitted but not recommended. Sessions were supervised by instructors with experience in small-sided football and matches played as 2 vs. 2; 3 vs. 3; or 4 vs. 4, depending on attendance. Games were played without goalkeepers and consistent measures were taken to ensure continuous play (e.g. immediate ball replacement if kicked out of play). Footwear was standardised by giving participants appropriate boots, which they wore at each training bout. Sessions took place outdoors on a grass surface or, in adverse weather and lighting conditions, either on an artificial surface (enclosed Multi-Use Games Area [MUGA] consisting of a quarter-size [38 x 18 m] 3g rubber crumb flood-lit pitch) or indoors, on a multi-use wooden gym surface. Pitch dimensions were between 15 – 25 m wide by 20 – 40 m long and two five-a-side goals (3.6 x 1.2 m) were used.

Before training a standardised warm up was performed, which consisted of 2 min submaximal shuttle runs and mobility exercises for the spine and lower limbs. During the submaximal exercise, participants completed levels 1-6 of the Yo-Yo Intermittent Endurance Level 1 Test (YYIE1) (Castagna et al., 2006), performing six 40 m sagittal runs with a 180 degree turn between each 20 m shuttle and 5 s active recovery between levels (i.e. after each 40 m run completed), in which participants walked 2 x 2.5 m around a cone. After the submaximal shuttle runs a standardised 1 min protocol of mobility exercises was performed, which included trunk flexion, extension and side-bending, and range of movement exercises for the hip, knee and ankle joints.

WBV Participants completed two 13.5 min sessions per week on a WBV plate (Galileo Sport, Novotec Medical GmbH, Pforzheim, Germany) with a minimum of 24 h between sessions. Participants were instructed to take off their footwear and stand on the plate, knees slightly flexed, with their bodyweight over the forefoot. The protocol consisted of a 3 min warm up at a frequency of 6 Hz with an amplitude of 2 mm, followed by 1 min bouts of the following actions in sequence: (1) isometric squat (30° knee flexion); (2) dynamic squat (alternating between 30° - 90° knee flexion); (3) functional pelvic floor muscle loading (alternating maximal anterior to maximal posterior pelvic tilt); 4) isometric squat (30° knee flexion); (5) global (whole spine) flexion and extension; (6) isometric squat (30° knee flexion); dynamic squat (alternating between 30° - 90° knee flexion); (7) functional pelvic floor muscle loading (repeat of previous actions). Participants were familiarised with all actions prior to their initial WBV training session. The actions described in bouts 1-4 above were performed during WBV at a frequency of 12 Hz throughout the 16 week training intervention; vibrational frequency for bouts 5-7 was ramped from 12 Hz (w 1-4), to 18 Hz (w 5-7), to 27 Hz in (w 8-16). Vibrational amplitude was also ramped incrementally from 1 mm (w 1) according to the following schedule: 1.5 mm (w 2-3), 2 mm (w 4-5), 2.5 mm (w 6), 3 mm (w 7-9), 3.5 mm (w 10), 4 mm (w 11-16). In addition in w 14-16 an external load was applied to the platform during bouts 1, 2, 5 and 6, which was increased from 4 kg (w 14), to 6 kg (w 15), to 8 kg (w 16). All training sessions were attended by a member of the research team, who supervised the actions performed and monitored participant safety when using the vibration plate.

4.2.8 BMD and body composition

BMD of the lumbar spine, bilateral proximal femora, neck of femur, total hip and total body, and total lean and fat mass, were measured using DXA (GE Lunar Prodigy, GE Healthcare, Bedford, UK) (see section 3.2.6).

Ratio of total fat mass (TFM) to total lean mass (TLM), and android obesity index (total android fat mass: total gynoid fat mass) pre- and post-intervention, were calculated from DXA measurements of these parameters.

4.2.9 Measurement of balance

Balance was assessed using the Flamingo test, as described in General Methods, section 3.8, paragraph 2.

4.2.10 Locomotor profile and exercise heart rate

SSFT Participants wore a global positioning system (GPS) unit (GPSports SPI Pro X, GPSports, Canberra, Australia) with a sampling frequency of 5 Hz, and a heart rate belt (Polar T34, Polar Electro Oy, Kempele, Finland), as described in section 3.5.1.

Velocity domains, computed using manufacturer software (Team AMS v. 1.5, GPSports, Canberra, Australia), were used to calculate distance covered in discrete locomotor activities (Randers et al., 2010). These included: standing (0 - 0.4 $km.h^{-1}$), walking (0.4 - 5 $km.h^{-1}$), jogging (5 - 7 $km.h^{-1}$), low-speed running (7 - 9 $km.h^{-1}$), moderate-speed running (9 - 11 $km.h^{-1}$), high-speed running (11 - 15 $km.h^{-1}$) and sprinting (> 15 $km.h^{-1}$).

Total distance covered, peak speed and high intensity running (sum of high-speed running and sprinting) and accelerations were extracted during data download (General Methods, Chapter 3, 3.5.2), along with minimum, mean and peak exercise heart rate (HR), and speed, which were automatically calculated by manufacturer software. Individual HR_{MAX} was determined to be the highest exercise HR recorded during SSFT sessions. GPS data were downloaded for outdoor sessions but were not obtained during indoor training. Data were split for each participant to create a separate file for submaximal exercise (2 min shuttles) and submaximal data split to examine HR during the final 15 s of submaximal exercise. A minimum of two sessions in early (weeks 1 - 4), mid- (weeks 7 - 10) and late- (weeks 13 - 16) phases of the study were analysed for each participant, to provide mean values for GPS data during these phases, and to account for match-to-match variation.

WBV Exercise HR during WBV sessions was recorded in ten participants using a HR belt (Polar T34, Polar Electro Oy, Kempele, Finland). Six participants assigned to WBV declined to wear the belt during WBV, but provided HR at rest at baseline, and on exit to the study.

4.2.11 Football activities

Outdoor sessions were filmed (Sony HandyCam, Sony Corporation, Japan) with the camera positioned at least 1.5 m behind a corner of the playing surface to capture the whole pitch with all participants in view. Video files were exported for visual analysis. Activity profile for each participant was examined at

baseline (session 1) and for a minimum of three sessions between weeks 1 – 4, 6 – 10 and at least two sessions in weeks 15 – 16. Episodes of the following categories of movement were counted: ball heading, jump, pass, shot, stop, tackle (shoulder), tackle (foot), turn: 0 - 90°, 91 - 180°, 181 - 270°, 271 - 360°, reception (air), reception (floor), block, sprint, run, sideways cut, backward walk, backward run. Activity total per session was calculated as the sum of actions in each category. An experienced, single rater analysed all videos of sessions, and in test-retest analysis of the same participants (10 x 13.5 minute repeats) no systematic differences were observed (CoV = 0 -12%).

4.2.12 Perception of effort for SSFT

A visual analogue scale VAS was used to rate perception of effort (see section 3.6.2) after the first session of SSFT. To retain response-sensitivity, rating of effort was not collected after every session; an average was calculated from VAS scores after a minimum of three sessions in early- (weeks 1 - 4), mid- (weeks 7 - 10) and late- (weeks 13 - 16) phases of the intervention.

4.2.13 Statistical approach

Participant data were tested for normality (Shapiro-Wilk test) and where found not to observe a normal distribution, data were rechecked for errors and decisions taken on a case by case basis, with regard to inclusion or exclusion of outlier data and statistical approach, as reported in results.

To compare responses to the intervention between groups, two-way mixed model analysis of variance (ANOVA) (SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) was used, with intervention group as the between group factor, and time as the repeated measure. Where indicated by a significant two-way interaction or main effect, post-hoc analysis, corrected for multiple comparison (Bonferroni), was conducted. For data collected within SSFT only (locomotor profile, football activities and perception of exercise effort), either paired t-tests (to compare pre- and post- intervention) or one-way ANOVA (for data collected at > 2 timepoints) were used, to compare responses during different phases of the football training intervention. In SSFT, in the untrained state at baseline, the relationship between bone biomarker peak response and derivatives of locomotor profile (accelerations and decelerations, sprint distance, total distance) were examined using Pearson's correlation coefficient, and r and p values reported.

Acute bone biomarker responses to exercise were examined in the exercise groups only, due to limitation of resources (assay cost). Separate two-way mixed ANOVAs were performed for each biomarker to compare responses in the untrained state, with time of sample as the within-subjects' factor (four levels: pre-, post-, 30 min post-, and 48 h post-exercise), and intervention group (two levels: SSFT and WBV) as the between-group factor. To compare the effect of chronic exercise on peak magnitude of biomarker response, data at baseline and after 16 weeks training were examined, timepoint of peak response identified, and magnitude of response calculated for each individual (delta peak response versus fasted pre-exercise). Two-way mixed ANOVAs were used to compare peak response to WBV and SSFT in the trained and untrained state. This approach was also adopted to compared peak venous blood lactate response to exercise, pre- and post- 16 weeks training.

Significance was set at $p < 0.05$.

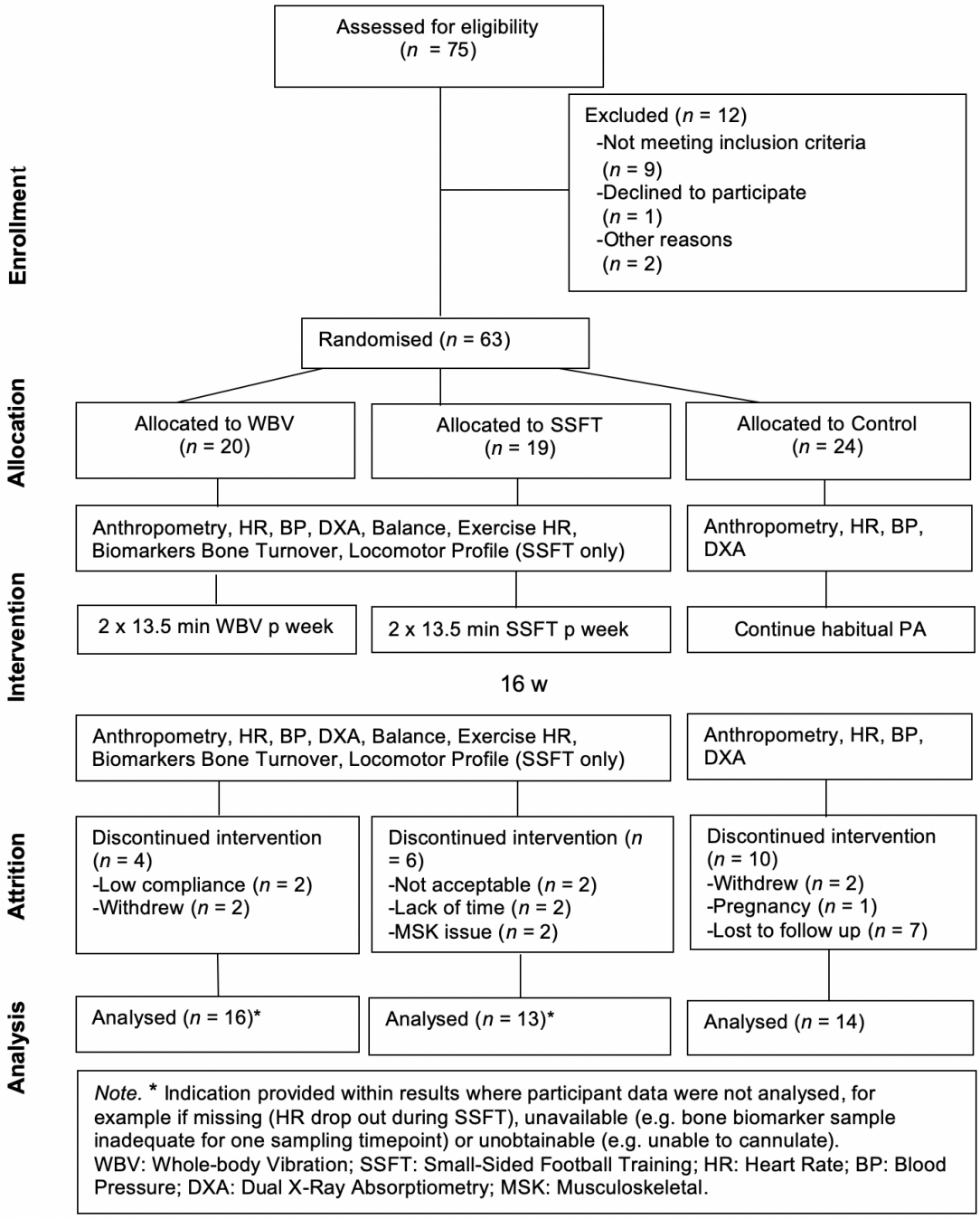


Figure 2 Consort Flow Diagram of Study Phases

4.3 Results

4.3.1 Participants

A total of 43 women (mean 39.1 ±4.8 y; range: 28 to 46 y) completed the study (Figure 2). According to interventional group, numbers completing were as follows: CON (*n* = 14); VIB (*n* = 16), SSFT (*n* = 13).

Baseline data for participants who completed the intervention were normally distributed, and no statistical differences were found between intervention groups (Table 1). Inspection of questionnaire data indicated none of the participants had experience of WBV or football, except one participant randomised to SSFT, who had played football at school.

4.3.2 Attrition, adverse events

Attrition, as a percentage of total participants recruited to each group who completed the intervention, was 42% in CON, 32% in SSFT and 20% in WBV. In total 20 women previously recruited either did not complete or withdrew from the study, for reasons described in Figure 2. In SSFT, one of the participants who withdrew cited Achilles tendon pain, and another low back pain, which these participants attributed to football training.

In SSFT a mean of 32 (±4) sessions were completed by participants during 16 weeks training (mean compliance 1.8 sessions per week). During the intervention, four participants reported a soft tissue (ST) injury: calf strain (n = 1); thigh strain (n =1); ankle sprain (n =1) and anterior hip strain (n =1), all of which resolved without medical intervention. Sessions missed due to injury were recorded, and participation in the intervention extended until 16 weeks training was completed (n = 2 participants) or, if more than four sessions were missed (n = 2 participants), two weeks additional training were completed to counter potential de-training effects, and sessions after this period were included as part of the intervention requirement.

There were no adverse events in WBV and 100% attendance and compliance with training was recorded.

Information from a questionnaire (see Appendix A1.1) completed by participants after 16 weeks indicated there were no changes in musculoskeletal health or dietary habits during the intervention period.

4.3.3 Heart rate and blood pressure

HR at rest significantly decreased by 3 ±2 bpm after 16 weeks (p = 0.048, η^2 = 0.153, main effect of time), no other main or interaction effects were found for body weight, BMI or blood pressure (Table 2).

Table 2 Effects of 16 Weeks WBV or SSFT on Cardiovascular and Anthropometric Characteristics (Mean ±SD)

Characteristic	Intervention Group						*p*		
	CON (*n* = 14)		SSFT (*n* = 13)		WBV (*n* = 16)		Group	Time	Group* Time
	0 w	16 w	0 w	16 w	0 w	16 w			
HR bpm	80 (±10)	73 (±8)	77 (±8)	73 (±8)	74 (±11)	72 (±11)	0.692	0.048	0.663
Systolic mmHg	117 (±14)	116 (±14)	120 (±17)	119 (±13)	123 (±13)	119 (±16)	0.838	0.309	0.321
Diastolic mmHg	75 (±9)	75 (±9)	77 (±11)	76 (±10)	77 (±9)	76 (±11)	0.962	0.533	0.900
MAP mmHg	89 (±10)	89 (±10)	91 (±13)	92 (±11)	92 (±9)	90 (±12)	0.915	0.607	0.477
Weight kg	68.6 (±13.9)	68.8 (±14.4)	67.9 (±10.2)	67.1 (±9.2)	72.2 (±13.5)	72.6 (±13.5)	0.551	0.882	0.373
BMI kg.m^2	24.9 (±4.9)	25.0 (±4.9)	24.9 (±3.8)	24.5 (±3.6)	25.2 (±4.5)	25.3 (±4.5)	0.940	0.551	0.382

CON:Control; SSFT: Small-sided football training; WBV: Whole-body vibration; HR: heart rate; MAP: Mean arterial pressure; BMI: Body mass index.

4.3.4 DXA and body composition

There was a tendency for total hip bone mineral density (BMD) to differ between groups after 16 weeks (p = 0.063; η^2 = 0.129). In SSFT, total hip BMD increased significantly from 1.028 ±0.143 g.cm^2 to 1.037 ±0.140 g.cm^2 (p = 0.042; adjusted for multiple comparison) and did not change in either WBV or CON (Table 3). Arm BMD reduced by 0.049 ±0.011 g.cm^2 (p < 0.001; η^2 = 0.332, main effect of time); no other main effect of time, group, or time by group interaction effects, were found for BMD in other regions of interest, or for total body (Table 3).

Ratio of total fat mass to total lean mass reduced (p = 0.050; η^2 = 0.093, main effect of time), decreasing in SSFT but not in WBV or CON (p = 0.037; η^2 = 0.153, time*group interaction) from 0.66 ±0.22 to 0.61 ±0.18 (p = 0.015, adjusted for multiple comparison). There was a tendency for lean mass to be increased after 16 weeks (p = 0.053, η^2 = 0.090, main effect of time), paired T-tests within group, adjusted for multiple comparison, showed that the increase in lean mass in SSFT (770.2 ±284.2 g) was statistically significant (p = 0.028) whereas pre- post- values were not significantly different within WBV and CON (p > 0.999).

The effect of the intervention on android fat mass tended to be different between groups (p = 0.062, η^2 = 0.130, time*group), reducing in SSFT by 171.1 ±74.7 g (p = 0.081) and in CON by 19.6 ±53.6 g (p > 0.999) and increasing by 52.3 ±66.9 g in WBV (p = 0.892).

Table 3 Bone Mineral Density, Lean and Fat Mass Pre- and Post- 16 Weeks (Mean ±SD)

	Intervention Group						ANOVA group * time	
Measure	**CON (*n* = 14)**		**SSFT (*n* = 13)**		**WBV (*n* = 16)**		***p***	***partial η²***
	0 weeks	**16 weeks**	**0 weeks**	**16 weeks**	**0 weeks**	**16 weeks**		
BMD Total	1.191 ±0.095	1.183 ±0.086	1.154 ±0.087	1.148 ±0.084	1.207 ±0.092	1.200 ±0.093	0.929	0.004
BMD L1-L4	1.214 ±0.142	1.212 ±0.154	1.184 ±0.169	1.189 ±0.169	1.244 ±0.190	1.241 ±0.190	0.557	0.029
BMD NoF	1.002 ±0.140	0.995 ±0.137	0.997 ±0.146	0.994 ±0.143	0.988 ±0.143	0.988 ±0.143	0.739	0.015
BMD Total Hip	1.041 ±0.133	1.044 ±0.135	1.028 ±0.143	1.036 ±0.140[a]	1.009 ±0.132	1.006 ±0.132	0.063	0.129
BMD Legs	1.247 ±0.103	1.251 ±0.103	1.245 ±0.101	1.242 ±0.101	1.260 ±0.107	1.255 ±0.101	0.603	0.025
BMD Arms	0.930 ±0.135	0.857 ±0.068	0.879 ±0.072	0.849 ±0.064	0.931 ±0.077	0.889 ±0.077	0.269	0.063
BMD Head	2.515 ±0.321	2.499 ±0.353	2.364 ±0.278	2.349 ±0.285	2.534 ±0.346	2.570 ±0.348	0.102	0.108
BMC Total	2.641 ±0.537	2.640 ±0.529	2.640 ±0.463	2.604 ±0.486	2.879 ±0.420	2.892 ±0.417	0.288	0.060
Mass Total	67.58 ±13.71	67.62 ±14.10	66.72 ±10.04	66.05 ±9.81	71.23 ±13.45	71.55 ±13.41	0.528	0.031
FM:LM	0.61 ±0.21	0.61 ±0.21	0.66 ±0.22	0.61 ±0.18*	0.69 ±0.20	0.69 ±0.19	0.037	0.153
FM Total	24.90 ±9.90	24.74 ±10.03	25.43 ±7.69	24.02 ±6.85	27.76 ±9.07	28.01 ±8.87	0.123	0.100
LM Total	40.04 ±4.43	40.25 ±4.87	38.66 ±4.64	39.43 4.37T	40.58 ±6.25	40.64 ±6.32	0.233	0.070
Gynoid F Total	4.84 ±1.70	4.81 ±1.77	5.28 ±1.31	5.11 ±1.27	5.64 ±1.34	5.67 ±1.32	0.483	0.036
Android F Total	2.19 ±1.06	2.17 ±1.05	2.05 ±0.85	1.88 ±0.78	2.19 ±0.93	2.24 ±1.01	0.062	0.130
AOI	0.44 ±0.15	0.45 ±0.18	0.38 ±0.10	0.36 ±0.09	0.38 ±0.11	0.39 ±0.12	0.129	0.095

Note. BMD: Bone Mineral Density (g.cm^2); L1-L4: Lumbar spine levels 1-4; NoF: Neck of Femur; BMC: Bone Mineral Content (kg); AOI: Ratio Android: Gynoid fat mass; Units: Mass Total, Fat Mass, Lean Mass, Gynoid Fat, Android Fat: kg; FM:LM, AOI: Arbitrary Units (AU).

[a] Significant increase in Total Hip BMD in SSFT after 16 weeks (p = 0.042).

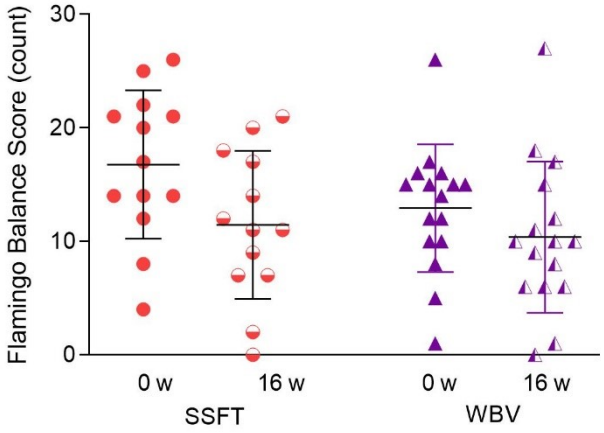
* Significant reduction in ratio of Total Fat Mass to Total Lean Mass in SSFT after 16 weeks (p = 0.015).

T Significant increase in Total Lean Mass within SSFT after 16 weeks (p = 0.028).

4.3.5 Flamingo balance test

After 16 weeks, balance score reduced in both SSFT and WBV (p = 0.003, η^2 = 0.280, main effect of time), there was no significant main effect of group or interaction effect (p > 0.05).

The number of re-stabilisations during 1 min of balancing reduced by 32% in SSFT (17±7 versus 11±7) and by 20% in WBV (13 ±6 versus 10 ±7) (Figure 3), and was not significantly different between exercise groups (p = 0.577).



SSFT: Small-Sided Football Training; WBV: Whole-Body Vibration.

Figure 3 Flamingo Balance Score Pre- And Post- 16 Weeks (Mean ±SD)

4.3.6 Blood metabolites

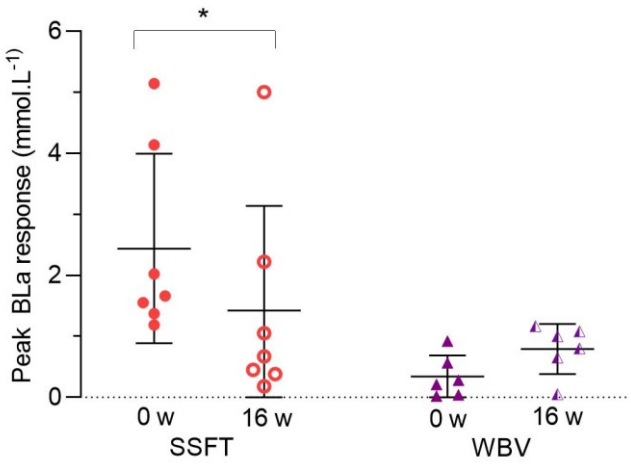
Lactate response to exercise

Untrained (0 wk) Venous blood lactate (BLa) concentration in response to exercise differed significantly between groups in the untrained state (p < 0.001, η^2 = 0.552, sample time*group). In SSFT, BLa increased from 0.6 ±0.2 mmol.L^{-1} to 3.2 ±1.4 mmol.L^{-1} immediately after exercise (p = 0.002) and reduced to 1.1 ±0.6 mmol.L^{-1} 30 min post-exercise, and was significantly reduced compared with BLa immediately after SSFT (p = 0.002). In WBV, BLa increased from 0.6 ±0.2 mmol.L^{-1} to 0.8 ±0.4 mmol.L^{-1} immediately after WBV and reduced to 0.5 ±0.2 mmol.L^{-1} 30 min post-WBV; BLa changes were not significant (p > 0.05).

Trained (16 wk) There was no significant time by group interaction effect on BLa response to exercise after 16 weeks training (p = 0.107, η^2 = 0.106, sample time*group), however BLa was significantly different across sampling timepoints (p < 0.001, η^2 = 0.602, main effect of sample time). BLa increased immediately after exercise to 1.1 ±0.2 mmol.L^{-1} in SSFT and 1.4 ±0.5 mmol.L^{-1} in WBV (p < 0.001) and significantly decreased to 0.8 ±0.5 mmol.L^{-1} in SSFT and 0.6 ±0.3 mmol.L^{-1} in WBV 30 min after exercise (p < 0.001).

Effect of 16 weeks training on peak lactate response

Inspection of data showed that peak lactate response in both exercise groups occurred immediately after exercise. Comparing delta BLa post- versus BLa pre-exercise a significant interaction effect was found for the effect of 16 weeks training on peak BLa response to exercise (p = 0.012, η^2 = 0.450, time*group interaction effect). In SSFT peak BLa response decreased from 2.4 ±0.4 mmol.L^{-1} to 1.4 ±0.5 mmol.L^{-1} (p = 0.021), whereas in WBV peak BLa response increased from 0.4 ±0.5 mmol.L^{-1} to 0.8 ±0.5 mmol.L^{-1} however, this was not statistically significant (p = 0.469). No main effects were observed.



SSFT: Small-SidedFootball Training; WBV: Whole-Body Vibration;
BLa: Venous Blood Lactate
* Significantly different 16 vs 0 w (p < 0.05).

Figure 4 Peak Lactate Concentration Pre- and Post- 16 Weeks (Mean ±SD)

Glucose response to exercise

Untrained (0 wk) There was a significant difference between groups in venous blood glucose (GLu) concentration in response to exercise (p = 0.004, η^2 = 0.327, sample time*group interaction effect). In SSFT, GLu significantly increased from 4.2 ±0.6 mmol.L^{-1} to 5.4 ±1.1 mmol.L^{-1} immediately after

exercise (p = 0.006) and reduced to 4.2 ±1.0 mmol.L^{-1} 30 min after exercise (p = 0.081), whereas in WBV, GLu did not change in response to exercise (4.1 ±0.4 versus 4.2 ±0.5 versus 4.1 ±0.4 mmol.L^{-1}, pre- versus post- versus 30 min post-exercise, respectively; p > 0.05).

Trained (16 wk) Glucose response to exercise differed between groups after 16 weeks training (p = 0.006, η^2 = 0.253, sample time*group interaction effect). In SSFT, GLu increased from 4.3 ±0.4 mmol.L^{-1} to 5.1 ±0.8 mmol.L^{-1} immediately after exercise (p = 0.014) and reduced to 4.6 ±0.4 mmol.L^{-1} 30 min after exercise (p = 0.081), a significant increase from basal (pre-exercise) (p = 0.036). In WBV, GLu did not change significantly across sampling timepoints (p > 0.05).

Effect of 16 weeks training on peak glucose response

As GLu after WBV did not change compared with pre-exercise in either the untrained (0 wk) or trained (16 wk) state, peak response was only examined in SSFT. A paired T-test was performed on peak GLu response (calculated as delta GLu immediately post- versus pre-exercise) at 0 wk and 16 wk, which showed peak response decreased in SSFT from 1.3 ±0.6 to 0.6 ±0.5 mmol.L^{-1} (p = 0.062) after 16 weeks training.

4.3.7 Biomarkers of bone turnover

Data for markers of bone formation (P1NP, OC) and resorption (CTX-1) were normally distributed in both WBV and SSFT, except for CTX-1 48 h post-exercise in one participant in SSFT in the untrained state. The decision was taken to retain this result as representing a valid measure in this participant, as a pattern of CTX-1 above the mean was seen across all timepoints in this individual.

Acute effects of exercise in the untrained state

P1NP In the untrained state, a significant difference between groups was observed for P1NP in response to exercise (p = 0.001, η^2 = 0.244, sample time*group). In SSFT, P1NP increased from 52.32 ±21.86 ug.L^{-1} prior to exercise to 58.38 ±21.96 ug.L^{-1} immediately after exercise (p = 0.014) and decreased 30 min after exercise to 49.25 ±18.54 ug.L^{-1} (p = 0.021), whereas P1NP in WBV did not vary significantly in response to exercise (p > 0.05). Plasma P1NP demonstrated significant variation across sample timepoints (p =

0.003, η^2 = 0.216, main effect of time) and was 18.33 ±6.23 ug.L^{-1} higher in SSFT than in WBV (p = 0.008, η^2 = 0.282, main effect of group).

OC Plasma OC was significantly elevated 48 h after exercise (p = 0.003, η^2 = 0.253, main effect of sample timepoint) and increased by 2.79 ±0.84 ug.L^{-1} compared with OC immediately post-exercise (p = 0.018) and by 2.67 ±0.84 ug.L^{-1} compared with OC 30 min after exercise completion (p = 0.026). Plasma OC was 5.58 ±2.46 ug.L^{-1} higher in SSFT (p = 0.033, η^2 = 0.190, main effect of group); no difference between groups was seen for the effect of exercise on OC (p = 0.427, η^2 = 0.036, sample*group).

CTX-1 Plasma CTX-1 did not vary in response to exercise (p = 0.116, η^2 = 0.104, main effect of sample timepoint) and response did not differ between exercise groups (p = 0.425, η^2 = 0.041, sample time*group). Unlike OC and P1NP, no significant main effect of group was observed for CTX-1 (p = 0.102, η^2 = 0.117, main effect of group).

Acute effects of exercise in the trained state

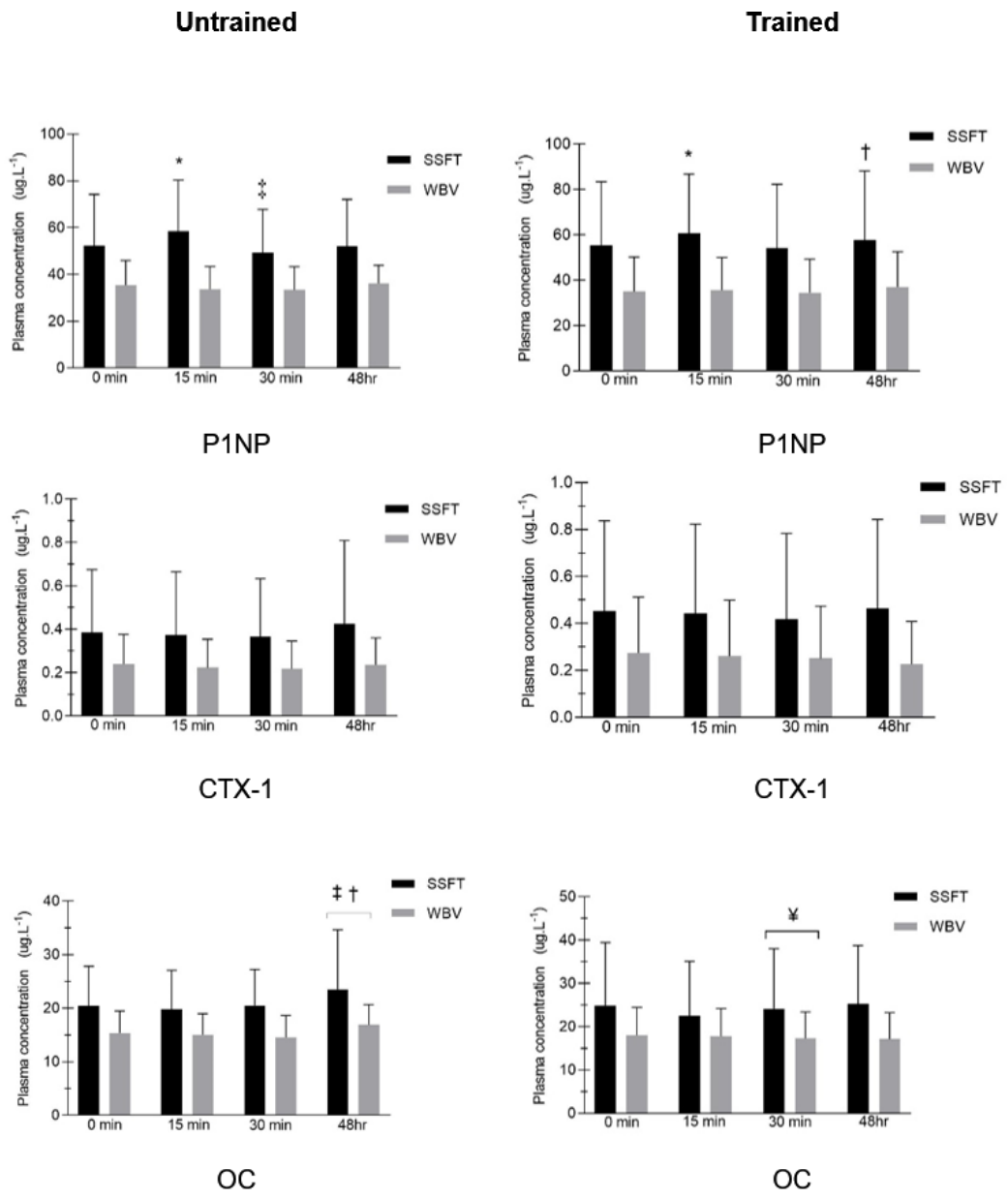
P1NP Plasma P1NP was higher in SSFT than in WBV (p = 0.022, η^2 = 0.216, main effect of group) and increased as a consequence of acute exercise (p = 0.003, η^2 = 0.240, main effect of sample time), peaking immediately post-exercise (p = 0.011), reducing 30 min post-exercise compared with immediately after exercise (p = 0.001) with a second P1NP elevation 48 h later (p = 0.039, 30 min post- versus 48 h post-exercise). The change in P1NP in response to exercise tended to be different between groups (p = 0.050, η^2 = 0.131, group*sample time), post hoc examination showed P1NP immediately after exercise increased by 5.51 ±2.04 ug.L^{-1} in SSFT (p = 0.163) and by 0.65 ±0.59 ug.L^{-1} in WBV (p > 0.999); 48 h after exercise P1NP was elevated by 3.60 ±2.25 ug.L^{-1} in SSFT (p = 0.887) and by 2.49 ± 0.83 ug.L^{-1} in WBV (p = 0.057), compared with results 30 min post-exercise (Figure 5).

OC Plasma OC varied significantly in response to exercise (p = 0.027, η^2 = 0.158, main effect of sample time) however responses were different between groups (p = 0.005, η^2 = 0.222, group*sample time). Whilst OC reduced in both groups immediately after exercise (p = 0.017), in SSFT plasma OC increased 30 min post-exercise compared with immediately after training (p = 0.048),

whereas in WBV plasma OC was lower 30 min post- compared with pre-exercise (Figure 5).

Unlike in the untrained state, there was no main effect of group ($p > 0.05$) for OC after 16 weeks.

CTX-1 As in the untrained state, no main effect of sample time, group or time*group interaction effect on plasma CTX-1 was observed for acute exercise in the trained state.



Note. Accessible to cannulation: Untrained: SSFT n = 11; WBV n = 13; Trained: SSFT n = 9; WBV n = 15. SSFT: Small-Sided Football Training; WBV: Whole-Body Vibration; P1NP: N-Terminal Propeptide of Type 1 Collagen; OC: Osteocalcin.
* Significant difference vs basal; ‡ Significantly different vs post-exercise; † Significantly different vs 30 minutes post-exercise; ¥ Significant difference between groups.

Figure 5 Biomarkers of Bone Turnover Trained vs Untrained State (Mean SD)

Chronic effect of exercise on background biomarker status

There was no difference between groups for the effect of 16 weeks training on fasted plasma P1NP, OC and CTX-1, sampled prior to exercise (p > 0.05, group* time interaction effect). No main effect of time was observed on background biomarker status, however resting plasma P1NP was 17.47 ± 6.72 ug.L^{-1} higher in SSFT than WBV (p = 0.015, η^2 = 0.207, main effect of group).

Table 4 Effect of 16 Weeks Exercise on Peak Bone Turnover Marker Response (Mean ±SD)

pBBTM ($ug.L^{-1}$)	State	Intervention Group						ANOVA Time*Group	
		SSFT			WBV				
		Basal	Peak	% change	Basal	Peak	% change	p	η^2
CTX1[a]	UT	0.39 ±0.29	0.43 ±0.38	6.1 ±24.1	0.24 ±0.14	0.24 ±0.13	6.4 ±29.3	0.944	0.000
	T	0.45 ±0.38	0.47 ±0.38	4.9 ±36.0	0.27 ±0.24	0.23 ±0.18	-1.0 ±54.4		
P1NP[a]	UT	52.23 ±20.84	58.44 ±20.94	15.8 ±15.6ᵀ	37.40 ±14.51	36.01 ±14.40	-3.7 ±7.1ᵀ	0.009	0.233
	T	54.82 ±24.07	59.89 ±22.40	11.2 ±9.4	34.71 ±14.76	35.41 ±13.96	3.9 ±8.5**		
OC[b]	UT	20.42 ±7.41	23.52 ±11.12	15.4 ±26.1	15.37 ±4.12	16.88 ±3.76	11.4 ±9.0	0.966	0.000
	T	24.85 ±14.59	25.29 ±13.45	3.5 ±12.0	18.06 ±6.43	17.15 ±6.07	-4.4 ±7.5		

pBBTM: Plasma Biomarker of Bone Turnover; CTX-1: C-Terminal Telopeptide of Type 1 Collagen; P1NP: N-Terminal Propeptide of Type 1 Procollagen; OC: Osteocalcin; UT: Untrained; T: Trained; SSFT: Small-Sided Football Training; WBV: Whole-Body Vibration.

[a] SSFT (n = 9), VIB (n = 15) participants accessible & provided patent 48h post-exercise sample.

[b] SSFT (n = 12), VIB (n = 16) participants accessible & provided patent post-exercise sample.

ᵀSignificantly different SSFT (UT) versus WBV (UT) ($p < 0.001$).

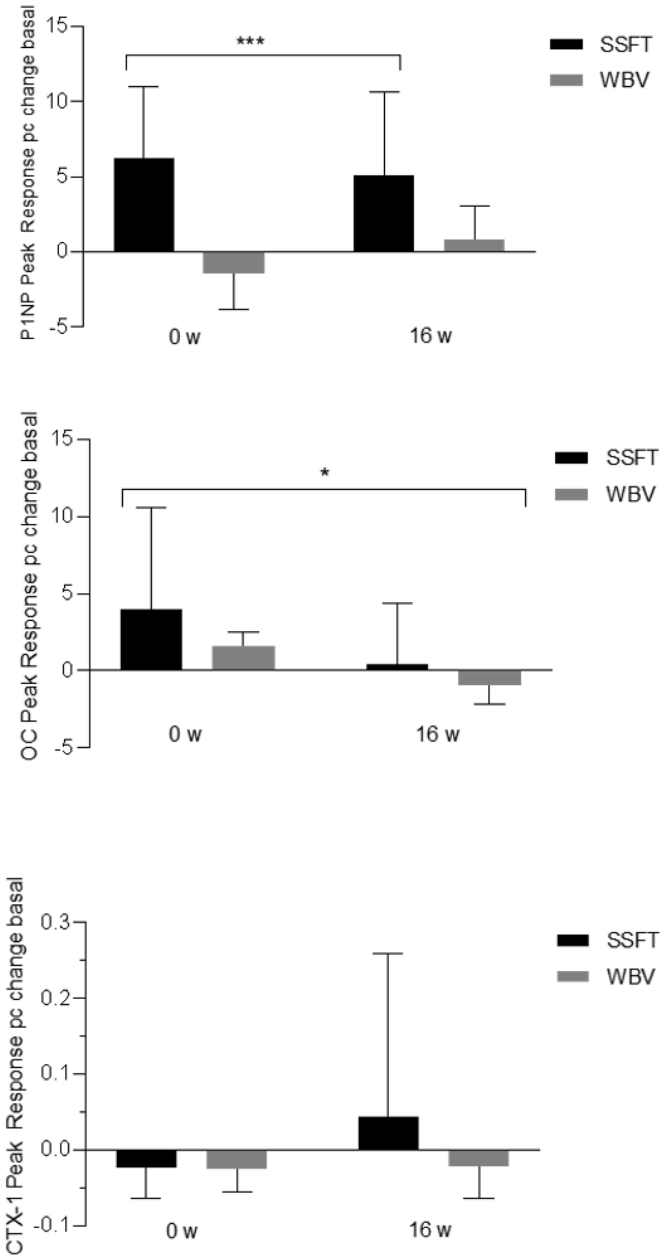
**Significant difference WBV (T) versus WBV (UT) ($p < 0.01$).

Chronic effect of exercise on peak bone biomarker response

P1NP Peak magnitude response immediately post-exercise was greater in SSFT compared with WBV by 5.96 ± 1.21 ug.L^{-1} ($p < 0.001$, $\eta^2 = 0.505$, main effect of group), however there was a trend towards an interaction effect ($p = 0.081$, $\eta^2 = 0.121$ time*group), whereby after 16 weeks training peak magnitude P1NP response post-exercise tended to decrease in SSFT and increase in WBV.

OC Magnitude of peak OC response 48 h post-exercise decreased in the trained compared with the untrained state ($p = 0.024$, $\eta^2 = 0.254$, main effect of time) and was reduced by 3.03 ± 1.23 ug.L^{-1} after 16 weeks ($p = 0.024$). No other main or interaction effect was observed.

CTX-1 No main or interaction effects were found for magnitude of CTX-1 response to exercise in the trained and untrained states.



SSFT: Small-Sided Football Training; WBV: Whole-Body Vibration; CTX-1: C-Terminal Telopeptide of Type 1 Collagen; P1NP: N-Terminal Propeptide of Type 1 Procollagen; OC: Osteocalcin

Figure 6 Effect of 16 Weeks Exercise on Peak Bone Turnover Marker Response

4.3.8 Exercise HR and locomotor profile

Small-Sided Football

After 16 weeks training, participants tended to perform more sprinting (p = 0.055) and high-speed running (p = 0.057), walked more (p = 0.012) and stood still less (p = 0.010) during small-sided football (Table 5). During the final training session, whereas total distance for all locomotor categories significantly increased (Table 6), mean heart rate decreased by 7 ±18 bpm (p = 0.199) and peak heart rate decreased by 7 ±9 bpm (p = 0.042). Training intensity (session HR_{MEAN} as a percentage of HR_{PEAK} from all available sessions) decreased from 83 ±7% to 80 ±6% (p = 0.197), reflecting reductions in mean and peak heart rate during SSFT in the trained state (Table 6).

Locomotor profile and bone biomarker responses at baseline

During the first session of SSFT (untrained state) absolute number of accelerations and decelerations above 1.4 $m.s^{-2}$ and osteocalcin peak response to exercise were inversely correlated (Table 7), no other association between accelerations and bone turnover marker (BTM) responses, or between total session distance, or total high-speed running distance, and peak BTM response was observed (Table 7).

It was not possible to analyse acceleration data after 16 weeks SSFT due to a manufacturer software malfunction, which caused data corruption during download of participants' acceleration files.

Submaximal exercise

No change was observed in mean, maximum or minimum heart rate during submaximal exercise comparing early, mid- and late- phases of the intervention (p > 0.05) and there was no change in exercise intensity, characterised as HR_{MEAN} as a percentage of HR_{MAX} (highest value from all training sessions) (p > 0.05).

Whole-Body Vibration

Heart rate did not change during WBV, at baseline or after 16 weeks training.

Table 5 Comparison of Locomotor Characteristics Before and After 16 Weeks SSFT (Mean ±SD)

Training state	Standing (0-0.4 k.h^{-1})	Walking (0.4-5 km.h^{-1})	Jogging (5-7 km.h^{-1})	LSR (7-9 km.h^{-1})	MSR (9-11 km.h^{-1})	HSR (11-15 km.h^{-1})	Sprinting (>15 km.h^{-1})
Untrained	1.8 ±1.2	436 ±50	185 ±40	156 ±52	106 ±35	60 ±36	2.3 ±4.8
Trained	0.9 ±0.6*	472 ±62*	190 ±40	167 ±60	129 ±66	167 ±259∓	12.7 ±16.1#

Units: metres; LSR: Low-Speed Running; MSR: Moderate Speed Running; HSR: High Speed Running.
Note. n = 12 participants provided GPS data; * Significantly different from Untrained ($p < 0.05$); ∓, #Tendency towards significant difference after 16 weeks (∓ $p = 0.057$; # $p = 0.055$).

Table 6 Total Distance, Speed and Heart Rate During SSFT (Mean ±SD)

Training state	Total distance (m)	HIR distance (m)	Mean speed (m.s^{-1})	Peak speed (m.s^{-1})	Minimum HR (bpm)	Mean HR (bpm)	Peak HR (bpm)	Training Intensity (HR_{MEAN} /HR_{PEAK})%
Untrained	946 (±123)	62 ±40	1.1 ±0.2	14.7 ±1.3	104 ±17	156 ±12	180 ±7	83 ±7
Trained	1073 (±101)**	179 ±272	1.3 ±0.1**	16.3 ±2.2*	101 ±14	149 ±10	174 ±7*	80 ±6

Units: metres; HIR: High Intensity Running (HSR+Sprinting).
Note. *n* = 12 participants provided GPS data; * $p < 0.05$; ** $p < 0.01$; Significant difference Trained versus Untrained.

Table 7 Accleration Profile During Baseline SSFT (Mean ±SD)

Training state	Acceleration			Deceleration			Total accelerations	Total decelerations
	0.6-1.0 m.s^{-2}	1-1.4 m.s-^{2}	>1.4 m.s^{-2}	0.6-1.0 m.s^{-2}	1-1.4 m.s^{-2}	>1.4 m.s^{-2}		
Untrained	37 ±41	25 ±5	22 ±11	62 ±16	40 ±12	24 ±8	83 ±17	126 ±31

Units: counts.
Note. *n* = 13 participants provided baseline accelerometry data.

Table 8 Association Between Peak Bone Turnover Marker Response and Locomotor Profile During Baseline SSFT

Bone Marker	Total distance (m)		HIR distance (m)		Accels. >1.4 m.s^{-2}		Decels. >1.4 m.s^{-2}		Total accels.		Total decels.	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
P1NP (*n* = 12)	0.127	0.256	- 0.057	0.862	-0.535	0.073	-0.503	0.096	-0.357	0.254	-0.344	0.273
OC (*n* = 11)	-0.031	0.928	-0.264	0.433	-0.602	0.049*	-0.616	0.043*	-0.325	0.329	-0.400	0.223
CTX-1 (*n* = 11)	-0.107	0.755	-0.376	0.255	-0.500	0.117	-0.583	0.060	-0.345	0.298	-0.403	0.219

P1NP: N-Terminal Propeptide of Type1 Procollagen; CTX-1: C-Terminal Telopeptide of Type 1 Collagen; OC: Osteocalcin; HIR: High Intensity Running (sum of High-Speed Running and Sprinting distance).
Note. * Significantly negatively correlated with accelerometry profile ($p < 0.05$).

4.3.9 Rating of exercise effort during SSFT

Perception of effort, quantified as VAS_{TOTAL} score (Arbitrary Units, [AU]), changed significantly during 16 weeks SSFT (p = 0.001, η^2 = 505, main effect of time), score increased by 7 ±1 AU between baseline and early-phase (weeks 1 - 4) of training (p < 0.001), tending to remain 5 ±1 AU higher in mid-phase (weeks 7 - 10) compared with baseline (p = 0.050), however perception of effort plateaued in the late- (weeks 13 - 16) compared with mid-phase of training (p > 0.999), and was not significantly different from baseline (p = 0.083).

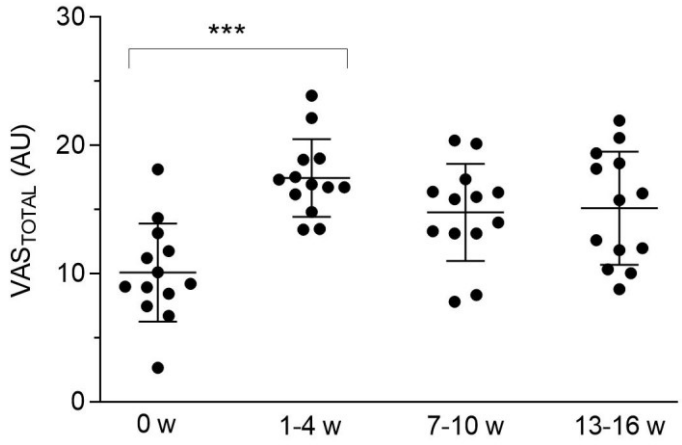


Figure 7 Perception of Exercise Effort During SSFT (Mean ±SD)

Football activities

There was a significant effect of phase of intervention on number of actions performed (p < 0.001, η^2 = 0.622), which increased from 104 ±18 at baseline to 128 ±24 in weeks 6 - 10 (p = 0.055) and 128 ±15 (p = 0.056) during the end phase of training (weeks 15 - 16), when total number of actions did not change compared with the middle phase of training (p > 0.999).

4.4 Discussion

This study found evidence of an osteogenic effect of brief, weekly training with small-sided football games (SSG) but not whole-body vibration (WBV) in sedentary premenopausal females. Total hip BMD measured by DXA significantly increased in women assigned to twice-weekly SSG for 13.5 min, whereas there were no changes in BMD after WBV prescribed at the same duration, or in controls. Alongside DXA results, an acute elevation in bone formation was observed at baseline immediately after SSG, with a further delayed elevation in formation 48 h after exercise in both exercise groups, however after 16 weeks acute biomarker response was attenuated after SSG but enhanced in WBV. Locomotor profile and exercise heart rate indicated there was a decline in training intensity during the final 4 week phase of SSG, unlike for WBV, which deployed a ramped protocol, results therefore suggest that sedentary females were accommodated to the osteogenic stimulus elicited by SSG after chronic training, which led to a reduction in acute bone formation response to a bout of exercise.

Whilst previous studies have demonstrated an osteogenic effect of recreational football, this is the first to provide preliminary evidence of localised bone adaptation in response to a low (27 min) weekly dose of training. For example, compared with the significant increase of 0.8 ±1.4% in total hip BMD within the football group in the present study, an increase of 1.3 ±0.3% in whole-body BMD was reported in sedentary premenopausal females after 4 months football played as 60 min twice SSG (Krustrup, Hansen, et al., 2010). Elsewhere, in a 15 week controlled intervention in sedentary premenopausal females that compared three weekly sessions of 60 min SSG with high- and moderate-intensity swimming at the same duration, bone mineral content (BMC) increased significantly after SSG by 1.7 ±1.9% at the femur and 2.4 ±2.9% at the trochanter, compared with swimmers and controls (Mohr et al., 2015).

Interestingly, alongside regional and whole-body evidence of bone adaptation in response to football, both Mohr et al., and Krustrup et al., reported significant changes in background bone biomarkers after 15 – 16 weeks training, an effect not observed for low-duration SSG after 16 weeks in the present study. It is possible that lower numbers of participants during football sessions in the present study (2 vs 2 compared with 5 vs 5 or 7 vs 7, as

reported by Mohr and Krustrup) could account for the absence of evidence of a constitutive osteogenic response. Speculatively, this may be attributable to differences in activity patterns and a comparative reduction in musculoskeletal loading, for example fewer bouts of extensive running and a reduction in competitive tackling, jumping and heading, during SSG with fewer participants.

In agreement with the evidence of constitutive effects of football on bone metabolism provided by Mohr and Krustrup and their colleagues in premenopausal females, in elderly males osteocalcin (OC) was found to have increased by 45% and P1NP by 41% after 4 months of 2 – 3 weekly sessions of 45-60 min football (Helge et al., 2014), which indicates that bone formation at rest was upregulated in men receiving the football intervention. Similarly, in men undergoing androgen deprivation therapy for prostate cancer, background OC increased by 7.3% alongside 0.4 – 1.0% increases in left and right femoral shaft and neck, after 32 weeks recreational football, ramped from an initial weekly training dose of 2 x 30 min to 2 x 60 min after 13 weeks (Uth et al., 2016). An RCT in inactive women, randomised to either 12 weeks football training or Zumba for 2 x 60 minutes twice per week, reported an increase of 11% (5.3 $ug.L^{-1}$) in background OC after 12 weeks in the football group, which was greater than the increase in OC after Zumba (3.3 $ug.L^{-1}$) (Barene, Krustrup, Jackman, Brekke, & Holtermann, 2014), and after 16 weeks football comprising twice weekly sessions of 4 x 12 minute games, sedentary premenopausal females demonstrated a significant 37% increase in resting plasma OC (Jackman et al., 2013).

Compared with what has been reported from studies of similar duration to the present, it is possible that at < 15 min per bout as prescribed here, training volume of SSG did not provide a sufficiently intense stimulus to exert constitutive effects on bone metabolism, which could explain the absence of any observed changes in background P1NP, OC and CTX-1 (a marker of bone resorption) after 16 weeks training. However, unlike the approach adopted in the previously cited studies, which measured biomarkers of formation and resorption at rest to characterise chronic effects of football on bone metabolism, the present investigation also examined bone biomarker responses to a single bout of training, and then characterised the effect of chronic training on this acute response. Accordingly, we were able to provide an alternative hypothesis to explain the evidence of localised bone anabolic effects of brief weekly SSG.

At baseline, plasma P1NP (pP1NP) response differed significantly between intervention groups, increasing by 15.8 ±16% immediately after SSG and returning to basal (pre-exercise) 30 minutes after exercise, whereas pP1NP did not change in response to WBV.

These results suggest that the increase in total hip BMD within the football group, in the absence of any evidence of increase in background bone biomarkers, could be accounted for by acute, transient elevations in bone formation, occurring in a quantal manner, as an episodic response to SSG exposure. According to this proposal, bone adaptation would occur in an integrative manner, through accretion of responses to discreet loading bouts, and in the present study was exhibited as a localised rather than systemic phenomenon. Therefore, even though duration of exercise was brief (13.5 min), compared to bouts of 45 - 60 min in previous football interventions, it was sufficient to elicit a transient increase in bone formation after football, but not after WBV, during initial training exposure. It may therefore be inferred that small-sided football games provided a greater acute stimulus to bone formation than WBV in untrained inactive females, and is in agreement with other evidence that in younger, healthy populations WBV provides an insufficient stimulus to promote significant morphological adaptation in bone and muscle (Dolny & Reyes, 2008; Lienhard, Vienneau, Nigg, Friesenbichler, & Nigg, 2017).

Mechanistically, it has been proposed that football actions, such as stops, tackles, jumps and brief bouts of sprinting, provide the stimulus to osteogenic effects attributed to participation in an interventional setting (Helge et al., 2010; Jackman et al., 2013; Krustrup, Hansen, et al., 2010). Time-motion analysis in untrained females has shown a high number (192 ±63) of these unpredictable loading actions during 60 min football training (Pedersen, Randers, Skotte, & Krustrup, 2009). Moreover, research in untrained males and females has shown the SSG format intensifies physiological demands of football participation (Randers et al., 2010), and in elite players SSG is shown to produce a higher index of football-specific technical actions than 11 vs 11 (Bujalance-Moreno et al., 2020). It may therefore be hypothesised, in accordance with a more fundamental explanation of bone adaptation provided by the mechanostat (Frost, 1987, 2004), that pP1NP was acutely elevated in untrained females immediately after initial SSG in response to mechanosensory

transduction of exercise-induced strains, which exceeded those prevailing in habitual PA. Conversely, absence of an acute bone biomarker response to initial WBV indicates the exercise stimulus, proposed to reside in vertical accelerations and reflex muscle contractions evoked by platform oscillations (Madou & Cronin, 2008; Rittweger, 2010), was sensed as inadequate to promote a bone anabolic response.

Whilst divergence in biomarker responses to exercise at baseline was speculatively attributed to differences in the mechanical strain environment of SSG and WBV, a tendency for the groups to differ in acute response after 16 weeks required further interpretation. Whereas after WBV magnitude of peak pP1NP response significantly increased (-3.8 ±7.1% vs 3.9 ±8.5% change on basal pP1NP pre- and post- 16 weeks), after SSG acute pP1NP elevation was retained, but was reduced (15.8 ±15.6% vs 11.2 ±9.4%, change on basal pre-exercise, 0 vs 16 weeks). These findings support a hypothesis that whereas ramping of the protocol increased the stimulus to osteogenesis elicited by WBV after 16 weeks, a training effect occurred in the football group, resulting in a decline in the intensity of the anabolic stimulus it provided. Furthermore, as an interval of 4 – 6 months is proposed for completion of bone formation (Clark, 2008) it is plausible that duration of the present intervention was at the lower limit for detecting bone accrual in response to exercise. Accordingly, it is possible to infer from biomarker responses to chronic training that longerterm exercise could produce an osteogenic effect of WBV, and further gains in BMD could also be accrued in the football group, were the interventional period to be extended.

Analysis of locomotor characteristics and perceptual rating of exercise effort supports a proposal of relative accommodation to the demands of SSG. During initial SSG at baseline, football-specific activities categorised as high-intensity efforts (Krustrup, Helge, et al., 2018) totalled 104 ±18 and peaked at 128 ±28 (sessions 8 - 10). In alignment with these results, after a significant increase in perception of demand during weeks 1 – 4, rating of effort plateaued during the final four-week phase. Converting football-specific actions per bout to a frequency, to enable comparison of present findings with those reported for longer bouts of SSG, results showed high-demand football actions increased in frequency from every 8.1 ±1.3 s in the untrained state to a peak of every 6.2

±1.0 s, with no further increase in frequency of actions observed during the final four week phase of the intervention. In comparison, a study that examined musculoskeletal effects of 60 min bouts of SSG, in premenopausal women of similar age to the present intervention group (37 ±2 y vs 39 ±2 y), reported a total of 1158 activity changes during 4 vs 4 and 1221 during 2 vs 2 (Randers et al., 2010), a rate of change in activity of 3.1 s and 2.9 s for 2 vs 2 and 4 vs 4, respectively. Alongside more frequent changes in high-intensity football actions than in the present study, descriptive values for HR_{MEAN} (161 ±6 vs 149 ±10), HR_{PEAK} (184 ±2 vs 174 ±7), and mean exercise HR as a percentage of peak exercise HR (81 ±8 vs 80 ±6), were higher in females in the study by Randers and colleagues than in sedentary females during the final phase of this intervention (Randers et al., 2010, ibid) , which adds evidential weight to the proposal that the stimulus of SSG as applied here was insufficiently ramped, and thus training adaptation was likely to plateau. Moreover, whereas BLa in response to initial training was significantly elevated above basal after SSG compared with WBV, after 16 weeks the interaction effect was obliterated, and magnitude of peak response was not significantly different from BLa at rest in either group. This adds further support to the proposal that physiological demands of SSG declined, and alongside the plateau in high-intensity actions, could explain the attenuation of peak pP1NP response after chronic training.

It should be stated that P1NP is not exclusive to bone tissue, and has been reported to be upregulated during enhanced Type-1 pro-collagen synthesis in muscle after eccentric quadriceps exercise (Crameri et al., 2004), and in the patella tendon after prolonged (36 km) running (Christensen, Dandanell, Kjaer, & Langberg, 2011). Therefore, the acute elevation in pP1NP observed in the present study could in part be attributed to upregulated pro-collagen synthesis in tendon and muscle. In accordance with this interpretation, the significant elevation after initial SSG could have occurred as a result of unaccustomed activity demands, particularly eccentric quadriceps loading during whole-body deceleration, landing and change of direction actions that characterise recreational football (Krustrup, Helge, et al., 2018; Sarmento et al., 2020) and other multidirectional team sports (Taylor, Wright, Dischiavi, Townsend, & Marmon, 2017). In accordance with this interpretation, the increase in P1NP after WBV in the trained state could be accounted for by greater muscle activity during lower limb and platform actions, increasing reflex-

mediated strains on muscle-tendon units, and as supported by the post-exercise increase in BLa in WBV after 16 weeks.

In support of the hypothesis of an acute effect of exercise on bone formation mediated by P1NP, a recent intervention in postmenopausal females reported significant increases in P1NP of 7.7 ±1.8%, 9.4 ±1.3% and 10.6 ±1.6%, after 60 repetitions of either countermovement, drop or diagonal jumps, respectively (Prawiradilaga, Madsen, Jørgensen, & Helge, 2020), responses which are lower than the acute 15.8 ±16.0% increase in pP1NP after initial SSG exposure in the present study. It is interesting to note that dispersal of P1NP results reported by Prawiradilaga et al., was much smaller than in the present study. Whereas the former study prescribed a standardised ('closed') action to deliver impacts and predominantly axial compressions, high-intensity actions during SSG were non-uniform ('open') and occurred in response to demands of play. It could therefore be hypothesised that variation in P1NP responses within participants assigned to SSG could be accounted for by differences in exposure to impacts during football activities and vertical accelerations associated with locomotor profile. In fact, Prawiradilaga and colleagues have demonstrated a dose-response relationship between ground reaction force (GRF) and P1NP post-exercise (Prawiradilaga et al., 2020), which suggests that intensity of impacts associated with vertically oriented actions could function as a multiplier of bone anabolic response. This is in agreement with observational evidence in premenopausal females of a positive association between BMD at the proximal femur and chronic exposure to strains exceeding 3.6 g (Jämsä et al., 2006), and for BMD at the hip to be positively associated with accelerations above 3.9 g within PA (Vainionpää et al., 2006). Although these latter studies reported bone adaptation in the hip region, where a significant localised increase was observed after 16 weeks SSG, osteogenic effects were attributed to exposure to higher magnitude accelerations in PA profile than were accessible to quantification with motion sensor technology used during SSG, which is a limitation of present results.

Whilst acceleration profile during initial SSG was successfully characterised, due to a manufacturer software malfunction acceleration data were not available after 16 weeks training. Using available data, we did not find evidence of a relationship between high-intensity actions and bone biomarker

peak response: high-speed running and sprinting distance at baseline, and after 16 weeks, and total accelerations and decelerations above 1.4 g at baseline, were not associated with peak magnitude pP1NP response. Nevertheless, it would be interesting in future to undertake orthogonal-specific deconstruction of acceleration data at higher thresholds, to quantify directional efforts during brief exercise, which could yield insight into potential relationships between vertical accelerations characteristics and bone biomarker responses.

As no changes, either acutely or for chronic training, were seen for resorption characterised by CTX-1, the bias of evidence in the present study is towards episodic, bout-dependent increases in bone formation as the candidate mechanism for exercise induced adaptation. However, responses for OC are more difficult to explain. In the untrained state, significant elevation in OC in both exercise groups 48 h after exercise may be indicative of a 'true', i.e. osteokine mediated response to bone loading (Lombardi, Perego, Luzi, & Banfi, 2015; Pagnotti et al., 2019), yet an osteogenic effect of chronic training was observed after SSG but not WBV, and only at the hip. As it has been proposed that P1NP sampled at rest is indicative of constitutive bone formation (Qi, Liu, & Lu, 2016; Szulc, 2018), it is possible that the delayed, second elevation in pP1NP 48 h after exercise could represent a 'true' bone formation response in the trained state, and account for the increase in total hip BMD in SSG, in the absence of an elevation in OC after chronic training. However, as magnitude of 48 h pP1NP elevation, which as percentage change on basal pP1NP pre-exercise was non-significantly elevated in both exercise groups, by 5.8 ±10.6% after WBV, and by 3.0 ± 10.9 % after SSG but did not exceed CoV for the assay (5.4 – 6.1%), variation within sampling as well as intra-individual variation cannot be excluded in interpretation of this result.

Accompanying 0.8 ±1.4% increase in total hip BMD after 16 weeks SSG, women assigned to football also demonstrated a significant 0.8 ±0.3 kg gain in total lean mass (TLM), whereas body composition did not change after 16 weeks WBV or in controls. Whilst no increase in whole-body BMD was found in any intervention group, magnitude of change in TLM after SSG is similar to what was reported after 4 months of a longterm (16 month) recreational football intervention in inactive premenopausal women, in whom TLM significantly increased by 1.0 kg after twice weekly SSG, alongside 1.3% increase in total

BMD (Krustrup, Hansen, et al., 2010), although volume of exercise was higher per bout (60 min vs 13.5 min) than in the present study. As muscle strength was not functionally assessed, increased TLM after chronic SSG cannot be considered in relation to a football-specific outcome, such as maximum knee extensor strength or peak power during vertical jumping, which has been demonstrated to increase after 14 weeks recreational football training at a twice weekly 60 minute dose in female subjects (Helge et al., 2010). However, present results are in accordance with evidence of significant gains in total and leg lean mass for recreational football participation in older (Luo, Newton, Ma'ayah, Galvão, & Taaffe, 2018) and middle-aged populations (Zouhal et al., 2020), which have been ascribed to intermittent high-magnitude mechanical strain derived from muscle-GRF interactions (Bujalance-Moreno et al., 2019; Krustrup, Helge, et al., 2018; Zouhal et al., 2020).

In SSFT, increased TLM was accompanied by a tendency for android FM to be significantly reduced by 0.172 ±0.075 kg compared with baseline, and for total FM (TFM) to be reduced by 1.4 ±0.7 kg. This finding is comparable to evidence of a reduction of 1 – 3 kg for 12 – 16 weeks recreational football in non-elite populations (Oja et al., 2015), at a higher session duration (60 min vs 13.5 min) than in the present study. Whilst the magnitude of reduction in TFM after 16 weeks SSG is within the 'clinically relevant' range reported for football training as an intervention in an obese population (Milanović et al., 2019), this finding may have additional relevance for potential bone outcomes as a result of exercise participation. Changes observed in TLM, TFM and android FM in SSFT are consistent with phenotypic adjustment towards a net reduction in adipocyte-mediated pro-inflammatory signalling, evidenced to be deleterious to bone in the osteosarcopenic elderly (Ormsbee et al., 2014) and obese (Cao, 2011).

Evidence is accumulating for depot-specific actions of adipose tissue (AT) (Guglielmi & Sbraccia, 2018), its role in metabolic and inflammation-mediated disease (Bastard et al., 2006; Hocking, Samocha-Bonet, Milner, Greenfield, & Chisholm, 2013; Lewitt, 2017; Wajchenberg, 2000), and the proposed relationship between adiposity and bone health (Cao, 2011). Increased truncal fat mass (TrFM) ('android obesity') has been implicated in elevated cardiovascular (CV) disease risk and metabolic derangement (Walker,

Marzullo, Prodam, Bona, & Di Blasio, 2014), and android-to-gynoid fat ratio shown to be strongly negatively associated with total hip BMD post-menopause (Da Shao et al., 2015; Fu et al., 2011), with TrFM found to be more negatively associated with femoral neck strength than TFM in both pre- and post-menopausal women (Kim, Lee, Kim, & Koh, 2017). Evidence of superior BMD localised to the hip for reduced TrFM aligns with the present finding of increased total hip BMD accompanying reduced android-to-gynoid fat ratio in SSFT, as a result of decreased android fat mass (gynoid fat mass did not change in any interventional group). The potential of PA in general, and of SSG specifically, to modify CV and metabolic disease risk profile is likely to depend, at least in part, on the capacity of the exercise stimulus to perturb the balance between fat and lean tissue and promote localised AT reduction, particularly in the trunk.

Despite evidence presented here of an osteogenic effect of SSG at a lower dose than previously implemented, alongside adaptations in lean and adipose tissue concordant with potential improvement in bone as a result of decreased systemic inflammation, advocacy of 'exercise as medicine' (Pedersen & Saltin, 2015) requires the patient to adhere to the prescription for benefits to be accrued. However, as a percentage of total participants recruited, attrition was 32% for SSG, 20% for WBV and 42% in controls, and the most frequently cited reason for leaving the study was lack of time to attend exercise sessions, even though session duration was brief. This indicates a requirement to consider format of administration in future interventions, to increase accessibility of exercise, as our results suggest premenopausal females may experience time poverty as a barrier to exercise.

4.4.1 Limitations

There were a number of limitations to the present study: whilst conditions for WBV were standardised, SSG training was undertaken at different times of day and on different surfaces (grass vs MUGA vs indoor gym), which could have affected the osteogenic effect of exercise as a result of differences in compliance for each surface, and variation in attenuation of impacts. Furthermore, as recruitment was slow and attrition higher for football than WBV, inability to batch-recruit successfully reduced the numbers available to play SGG, which could have altered physiological demands of training, for example

by increasing or decreasing pressure to perform high-intensity football actions, with consequent implication for the stimulus to osteogenesis provided by participatory actions. Finally, as not all participants were accessible to blood collection at each sampling timepoint, or provided samples suitable for assay at every level of collection, data sets for comparison of the chronic effect of exercise on peak bone biomarker responses, and responses pre- and post-intervention, were not representative of all participants who completed the intervention. Furthermore, background biomarkers of bone formation and resorption were sampled in participants who received exercise as an intervention, but not in controls, due to limitation of resources, potentially limiting generalisability of results.

4.4.2 Conclusion

Evidence from this study suggests that whilst small-sided football at low duration can provide an acute stimulus to osteogenesis, this effect may be attenuated if training intensity plateaus, therefore in future interventions with brief, team sports activities, ramping to retain sensitivity to the stimulus, or elicit an effect for longerterm training, should be undertaken, as demonstrated by bone biomarker and blood lactate results after chronic WBV. Reducing exercise area, as shown for SSG compared with regular football, could increase the frequency of high-intensity actions proposed to provide the signal to bone adaptation, thereby intensifying the stimulus, and this could be examined in future interventions by imposing further constraints to exercise area. Whilst this study did not find evidence of a relationship between high-intensity actions and bone biomarker responses, this could be better described in future studies by characterising direction and magnitude of accelerations, to elucidate whether potential osteogenic effects exhibit threshold and orthogonal dependency. Finally, to increase acceptability and reduce perceived barriers to exercise associated with limited time, future implementation of brief exercise should consider how delivery may be optimised to enable premenopausal women to access PA in an interventional setting.

5 Development of Brief Diverse Movement High-Intensity Interval Training (DM-HIIT): The Grid Feasibility Study

5.1 Introduction: background and rationale

The requirement to attend externally scheduled training presents a barrier to exercise in premenopausal women, who may have limited time to travel and participate in structured exercise, due to work and family commitments (Ball, Salmon, Giles-corti, & Crawford, 2006). However, adherence to prescribed exercise under free-living conditions is vulnerable to inaccuracies in self-reporting (Dowd et al., 2018) and effectors of the exercise stimulus, particularly intensity and duration of self-regulated bouts, are inaccessible to direct monitoring (Colley et al., 2008).

Previously, in a randomised controlled study in sedentary premenopausal women, we examined the physiological demands and effects on bone of 16 weeks exercise, and compared brief (13.5 min) twice weekly sessions of either whole-body vibration (WBV) or small-sided football games (SSG). Drop out, as a percentage of recruited participants, was 34% for SSG and 20% for WBV, and 42% of controls did not persist with the study. In informal feedback, participants who dropped out of SSG cited issues in obtaining childcare, lack of time, and unacceptability of SSG as a form of exercise, as reasons for not persisting. Amongst participants who completed the football arm of the study, some volunteered feedback that they did not enjoy SSG, and others expressed the requirement to travel to the training venue for a brief bout of exercise as "*high effort-low reward*" (unsolicited participant comment). Low persistence and acceptability for SSG in brief prescription was not expected, given evidence of acceptability and high compliance with recreational football in this intensive format (Mohr et al., 2015), and of positive psychological effects, as a result of heightened flow, for regular SSG participation (Krustrup, Aagaard, et al., 2010).

It was speculated that an exercise format which was flexible, so that participants could schedule training to accommodate professional and family commitments, and which replicated the physiological demands of team sports yet could be practised independently, would have greater acceptability and promote higher compliance in sedentary females. If, as has been proposed,

characteristic SSG actions, such as intermittent bursts of running, hopping and jumping, are the effectors of musculoskeletal (MSK) adaptations demonstrated for recreational football in general populations (Krustrup, Helge, et al., 2018; Milanović et al., 2019), it was hypothesised that deliberate prescription of these actions could elicit MSK adaptation, independently of the team sport environment, if conditions of exercise intensity could be replicated.

At amateur and professional levels of participation, significant elevation in whole-blood lactate (BLa) concentration and exercise HR, alongside high perception of effort, have been shown for SSG (Dellal, Hill-Haas, Lago-Penas, & Chamari, 2011). There is evidence of improvement in functional outcomes, particularly repeated sprint ability (RSA) and change of direction (COD) capacity (Bujalance-Moreno et al., 2019), as a result of regular participation. It may be proposed therefore that adaptations demonstrated for SSG are at least partly attributable to the increase in density of high-demand actions, an observed effect of reducing pitch dimensions (Hill-Haas et al., 2011). For example, higher BLa has been reported in teamsport athletes after sprinting bouts that included COD compared with straight line actions (Buchheit, Haydar, Hader, Ufland, & Ahmaidi, 2011; Dellal et al., 2010), which suggests COD imposes a higher physiological load in a frequency dependent manner. In order to exploit this phenomenon and design conditions of frequent COD, it was proposed to constrain exercise area to a square marked out on the floor and referred to as ‘the grid’, in which participants would perform team sport activities, such as jumping, landing, lateral cutting and short sprints. It was speculated that restricting exercise area would increase whole-body COD and consequently decelerations and accelerations. Encountering the limit of the grid, participants would be required to change direction, for example by performing a whole-body rotation or reversing a progressive action. An audio-visual format, which could be streamed and accessed freely, was used for exercise delivery and to provide a visual cue to appropriately increase or decrease performance intensity.

In a team sport environment such as handball, evidence from time-motion analysis suggests frequency of high-intensity actions depends on phase of play, possession, and positional role (Karcher & Buchheit, 2014) and in court based sports often occurs in response to chaotic movements of the ball (Sweeting, Cormack, Morgan, & Aughey, 2017). As these conditions could not

be reproduced during home-training for a participant exercising alone, it was decided to regulate intensity externally by prescribing three temporal epochs, progressing from low- through intermediate- to highest-intensity, initiated by audio-visual cues during the training bout. A similar approach was first examined in a 7 week intervention in amateur male and female runners, who replaced usual training with a regimen of 10 – 20 – 30 s bouts, corresponding to low-, moderate- and high-speed running, in three (ramped to four) blocks of 5-min running, interspersed with 2 min rest (Gunnarsson & Bangsbo, 2012). Favourable effects on systolic blood pressure and lipid profile were observed in the training group compared with controls and VO_{2MAX} significantly improved, despite ~50% reduction in training volume (Gunnarsson & Bangsbo, 2012). These findings have been subsequently eproduced in a larger group of recreational runners (Gliemann, Gunnarsson, Hellsten, & Bangsbo, 2015). An advantage of applying the 10 – 20 – 30 s concept within bouts of team sports actions, as proposed for the present protocol, was that it would permit participants to self-regulate exercise intensity, thereby accommodating differences in baseline training status, which it was speculated might increase acceptability in a sedentary population.

In order to pursue the principal investigative theme of examining the effects on bone of brief, multidirectional exercise, movement content was chosen according to the hypothesis that non-uniform, intermittent sports actions have greater osteogenic potential than those that deliver more predictable, cyclic cycles of strain. For example, higher BMD has been reported in young, female artistic gymnasts compared with rhythmic gymnasts, at skeletal sites loaded during high-impact actions performed by the former class of athletes (Vicente-Rodriguez et al., 2007). In contrast to the vaulting, landing and high-intensity ballistic manoeuvres that characterise artistic gymnasts' performance code, their rhythmic counterparts execute movements that are typically smooth and monotonic (i.e. of uniform cadence), which suggests that brief, explosive actions provide a greater anabolic stimulus to bone than those that are continuous and metrically low in variation. Furthermore, it has been speculated that loading environment and even performance surface and footwear, influences osteogenic effects of exercise, with lower BMD in rhythmic compared with artistic gymnasts partly attributed to greater dampening of impacts on the yielding performance mat (Vicente-Rodriguez et al., 2007).

Proposal of a continuum of osteogenic potential, influenced in a volume-dependent manner by characteristics such as cadence, impact and intermittence, is supported by cross-sectional evidence in female athletes, who demonstrate phenotypic differences in BMD parameters. For instance, in collegiate females, acquatic athletes displayed lower pelvic, leg and whole-body BMD compared with runners, who in turn had lower whole-body and regional BMD than team sport athletes from softball, field hockey and soccer, whereas gymnasts demonstrated highest whole-body and regional BMD (Mudd, Fornetti, & Pivarnik, 2007). This finding is further supported by cross-sectional evidence in elite females of greater BMD in the spine and proximal femur in non-acquatic, compared with acquatic, athletes and age-matched (18 y) controls (Bellver et al., 2019). Sports-specific differences in whole-body and regional BMD have been further substantiated in cross-sectional examination of young female soccer and handball players and swimmers: BMD was significantly greater, at sites loaded during exercise, in girls who participated in weight-bearing team sports that featured intermittent actions and ground impacts, than in swimmers and non-exercising controls (Ubago-Guisado et al., 2015). However, whilst these findings suggest impacts are a significant determinant of bone anabolic effects for longterm exercise, interventions aimed at uncovering the relationship between impact exposure (dose) and bone response (effect) must consider how haphazard features of exercise can be standardised to interrogate potential dose-reponse relationships. Intermittent exercise such as team sport is described as an open-code activity (Vaeyens, Lenoir, Williams, & Philippaerts, 2008), and time-motion analysis shows high-intensity participatory actions occur unpredictably. For example in professional basketball, a court-based team sport, actions imposing high physiological demands arise in response to pressure of play (Stojanović et al., 2018), and in elite football, a field-based team sport, reflect positional demands (Bradley et al., 2009). Therefore, in an interventional setting, using choreography to include impacts in a scheduled, repeatable manner, could be an approach to standardising exposure through controlling exercise content.

It may be also be inferred, from cross-sectional evidence in athletes, that as well as sports actions, which are code-specific, periodicity- i.e. the inclination of typical performance movements towards either cyclic (stereotypical and repetitive) or acyclic (non-uniform, unpredictable) end points- may be a

determinant of osteogenic potential. Mechanistically, it may be hypothesised that non-uniform actions, which vary in tempo, generate accelerations, arising in a rate-specific manner from changes in speed. Exposure to high-magnitude accelerations in daily PA is positively correlated with areal hip BMD and improved femoral bone geometry (Ahola et al., 2010), and in premenopausal (35 – 40 y) females, has been shown to be predictive of greater anabolic effects on bone after chronic (1 y) exposure to impact exercise (Vainionpää et al., 2006). Therefore deliberate, brief prescription of intermittent activities, to provide a focussed acceleration dose, could enable the relationship between a controlled exposure to the proposed osteogenic signal and bone outcomes to be examined. However, this approach has not been previously undertaken in premenopausal females. It was therefore proposed to include diverse actions in the exercise protocol with an emphasis on vertical take-off and landing, particularly during the 10 s phases of high-intensity performance, according to meta-analytic evidence supporting site-specific anabolic effects of jumping in premenopausal females (Zhao et al., 2014). It was hypothesised that constraining exercise to the floor grid, in addition to increasing COD, would raise the osteogenic potential of exercise by disrupting monotonic execution of participatory actions, as an effect of braking and re-initiating actions at the edges of the grid. This is in accordance with the proposal that frequent accelerations and decelerations during team sports provide a mechanical stimulus to bone anabolism in a recreational setting (Sarmento et al., 2020).

A further interventional aim was to repurpose a tested exercise format and apply it in a novel context, to examine its potential to amplify effects of exercise on bone. Whilst high intensity intermittent-exercise training (HIIT) has been demonstrated to benefit a range of cardiovascular and metabolic health outcomes (Gibala & McGee, 2008; Grace et al., 2018), investigations have usually applied HIIT using cyclic, continuous actions, such as running (Menz et al., 2019) or cycle ergometry (Kong et al., 2016). Therefore, adopting a HIIT approach but using non-sagittal, diverse exercise actions, which is not represented in the literature, could offer novel insight into the effect of HIIT as an intervention for bone health.

DXA is the gold standard for measuring bone, as it has demonstrated high precision and extensive population-based data provide reliable reference

ranges (Blake & Fogelman, 2007). However, DXA lacks accessibility, particularly for field testing, and there is evidence that calcaneal quantitative ultrasound (QUS) is positively correlated with DXA, for instance, in a seven year follow-up study in postmenopausal females with osteoporosis (Trimpou, Bosaeus, Bengtsson, & Landin-Wilhelmsen, 2010). Lower specificity for QUS, despite similar sensitivity to DXA, has limited its exclusive use as a diagnostic tool in clinical settings (Manhard, Nyman, & Does, 2017). In contrast, to assess the effects of interventions on bone, QUS has advantages over DXA: it is portable, quick to perform, inexpensive and does not involve ionising radiation. Moreover, as calcaneal bone is predominantly trabecular, QUS is optimised to provide site-specific measurement of bone adaptations to loading, particularly for interventions utilising vertical impacts and multidirectional activities. For example, QUS has been used in a cross-sectional design that compared calcaneal bone stiffness in court-based athletes with non-exercising controls (Bravo-Sánchez, Abián-Vicén, Jiménez, & Abián, 2020), and a meta-analysis concluded QUS was a sensitive method for quantifying bone changes in diverse age groups in response to exercise interventions (Babatunde & Forsyth, 2013).

Alongside direct measurement techniques such as QUS and DXA, biomarkers of bone turnover have been used to assess the effect of pharmacological intervention on bone metabolism in patient populations (Szulc, 2018). Their use has been extended to characterise effects of exercise in different populations, for example, to compare high-intensity swimming and football SSG in females (Mohr et al., 2015), to assess longitudinal effects of different modes of exercise in adolescent males (Vlachopoulos et al., 2017), and to describe responses to recreational football in elderly males (Helge et al., 2014) and aerobic exercise in postmenopausal females (Wen, Huang, Li, Chong, & Ang, 2017). Despite a body of research into cardiometabolic effects of HIIT, bone metabolic responses, particulary effects of non-sagittal loading during HIIT on background bone formation and resorption, are unknown. As HIIT protocols are typically brief, compared with moderate intensity and continuous exercise regimens, evidence of anabolic effects on bone metabolism for exercise in a short-duration format has the potential to be well received, as lack of time is cited as a significant barrier to exercise in the general population (Roloff et al., 2020).

Therefore, the principal aim of this investigation was to conduct a 12 week feasibility study to examine acceptability and compliance with brief, diverse exercise in sedentary females, using a novel HIIT programme to deliver team sports actions in a format permitting flexible, autonomous participation. According to the hypothesis that reducing exercise area increases COD and reduces monotonic performance, we proposed to quantify the effects of accelerations, decelerations and prescribed vertical actions on bone, using QUS to measure calcaneal stiffness, and biomarkers of bone turnover to assess background bone metabolion.

5.2 Methods

5.2.1 Participants

Participants were recruited through poster campaign in Exeter and Somerset. A power calculation was not undertaken prior to recruitment, on the basis that the primary outcomes under consideration (feasibility and compliance) were being assessed for a novel exercise method, and therefore power to detect meaningful change in a quantified outcome could not be calculated from existing, peer-reviewed data.

In total, twelve women were recruited, eleven of whom met the following inclusion criteria:

- female
- aged 35 – 55 y
- not participating in more than 150 minutes moderate intensity exercise per week
- not pregnant or attempting to become pregnant
- generally healthy

Participants were excluded if answering ‘yes’ to one or more of the following questions:

- Have you experienced an episode of severe lower back pain in the previous 6 months?
- Have you had a musculoskeletal injury affecting the lower limb (foot, ankle, knee, hip) in the previous 6 months?

- Do you currently have neuropathy or experience nerve pain in the lower limbs?

All data gathering procedures, and the exercise training schedule, were explained to participants prior to being accepted onto the study and they were informed that they were free to withdraw, without giving an explanation, at any point during participation. Those accepted onto the study gave written, informed consent to participate and the study was approved by the Sport and Health Science ethics committee at the University of Exeter (REF 2013/690; see Appendix A2.1).

5.2.2 Schedule for data collection

Data were gathered at baseline and after 12 weeks training, as described in sections 5.2.3 to 5.2.12.

5.2.3 Venipuncture

Participants arrived early (8 a.m.) in a fasted state for venipuncture (see General Methods 3.3.1); for each participant, a venous blood sample was drawn into two yellow top serum vacutainers and then a single grey NaFluoride tube, according to the procedure described in General Methods 3.3.1 (paragraph 4). Serum samples were prepared as described (see General Methods 3.3.2) and placed in storage at -80 °C for subsequent analysis; the NaFluoride tube to provide a fasted glucose sample was immediately placed on ice to prevent sample degradation.

5.2.4 Biomarkers of bone turnover

Serum samples were subsequently analysed for N-terminal propeptide of Type-1 procollagen (P1NP) and osteocalcin (OC), biomarkers of bone formation, isoform 5b of tartrate-resistant acid phosphatase (TRAPcP 5b) and C-terminal telopeptides of Type-1 collagen (CrossLaps, [CTX-1]), biomarkers of bone resorption. This was undertaken by enzyme linked immunoassay (Human P1NP ELISA USCN Life Sciences, USA; N-mid osteocalcin ELISA; TRACP5b ELISA; and Crosslaps CTX: CTX1 ELISA Immunodiagnostic Systems, UK) at the laboratory of Sport and Health Sciences, University of Exeter, UK.

Manufacturer CoV (mCoV) was stated as 5.5 - 9.2%; 2.5 - 10.9%; 2.7 - 5.1%; and < 10%; for inter-assay mCoVs; and 6.0 - 13.9%; 1.7 - 3.0%; 1.3 -

2.2% and < 10%; for intra-assay mCoVS, for TRAPcP 5b; CTX-1; OC and P1NP, respectively.

5.2.5 Fasted glucose

Whole-blood glucose in the fasted state was assessed from the sample collected in a grey-topped NaFluoride tube, as described in General Methods, section 3.4.1.

5.2.6 Anthropometric data collection

Participant body mass (kg) and height (m) were measured (see General Methods 3.2.1 and 3.2.2) and BMI calculated from an average of three consecutive measurements (Table 9).

Table 9 Participant baseline characteristics (Mean ±SD)

Characteristic	Diverse Exercise Group (*n* = 11)
Age y	46.3 ±6.6
Height m	1.66 ±0.06
Weight kg	62.7 ±9.6
BMI $kg.m^2$	22.7 ±2.8
Resting HR bpm	74 ±9
Systolic BP mmHg	128 ±11
Diastolic BP mmHg	86 ±10
MAP mmHg	100 ±10
SI L (AU)	95 ±19
SI R (AU)	96 ±19
BUA R $dB.MHz^{-1}$	114 ±17
SOS R $m.s^{-1}$	1572 ±33
Ca FFQ $mg.d^{-1}$	1312 ±622
Flamingo Balance	6 ±5

BMI: Body Mass Index; HR: Heart Rate; BP: Blood Pressure; MAP: Mean Arterial Pressure; SI: Calcaneal Stiffness Index; AU: Arbitrary Units; BUA: Broadband Ultrasound Attenuation; SOS: Speed of Sound; Ca FFQ: Daily Calcium consumption.
Note. Flamingo Score represents restabilisation counts.

5.2.7 Heart rate and blood pressure at rest

Participants sat quietly for ten minutes before heart rate and blood pressure were measured at rest, and estimation of MAP calculated (see General Methods section 3.2.5; Table 9).

5.2.8 Quantitative ultrasound of the calcaneus

A quantitative ultrasound scan (GE Lunar Achilles Insight, Bedford, UK) was performed on the left and right calcaneus, as described in General Methods, 3.2.8.

5.2.9 Measurement of balance

Balance was assessed using the Flamingo test, as described in General Methods, section 3.8, paragraph 2.

5.2.10 Exercise protocol

Background to protocol design The author selected exercise actions to be deployed during grid training based on 15 years' experience of movement coaching in elite team and aesthetic sports codes (football, rugby, cricket and dance). Ecological evidence provided by extensive, first-hand supervision of athlete rehabilitation has underlined for the author the importance of retraining explosive sagittal and frontal plane actions, particularly those involving dynamic reversal from eccentric to concentric muscle activity in the lower limb, to enable successful return to play and optimal performative gains after injury. According to the rationale that high-demand activities represent the most potent drivers of musculoskeletal adaptation, these were prioritised for inclusion in choreography. The author also drew on considerable experience of multidisciplinary (MDT) 'backroom' team problem solving, for performance optimisation and injury prevention, and therefore the cutting, rapid striding, changes of direction and repeated flighted transitions, requiring hip range of motion, torque production and high-force ballistic lower limb actions, reflect movements used extensively during successful return to play (RTP) and performance optimisation protocols, in an MDT scenario. Whilst these had been ecologically deployed as it were a priori, in an athlete rather than non-athlete population, the author has also had 20 years clinical experience outside elite sport, and drew on content trialled successfully in rehabilitation programmes within a non-elite setting. The capacity to perform slow, controlled movements, as demonstrated within dance codes, alongside dynamic flighted activities, more typical in teamsport, which incorporate impact phases and ground reaction forces, were guiding principles in selecting actions used in the choreography. The author also looked at fundamental movements performed

during play, such as skipping, hop-scotch and tag, as these evoke whole body actions and solicit ground reaction forces, which were surmised to have osteogenic potential within a pre-determined choreography. These actions were then speculatively combined by the author, within an experimental template, and practised using different sized areas of floorspace, to assess subjectively the effect on heart rate and self-perception of exercise effort, before deciding on a standardisation of grid dimensions, based on foot length, for the feasibility study presently described.

Protocol implementation During supervised training at baseline, and after 4, 8 and 12 weeks training, participants locomotor characteristics and exercise HR were assessed, using a HR belt and GPS unit, fitted as described in General Methods, 3.5.1, and data were downloaded, using manufacturer software (General Methods, 3.5.2), and analysed to provide information about distance covered in locomotor categories assessed.

The training took place in a limited floor space (‘grid’) laid out as a square normalised to participant by converting shoe size (EU) to a dimension corresponding to foot length (International Shoe Size Chart; web reference 1) multiplied by 12. Materials and instructions were given to each participant to enable them to set out the grid using a pre-cut length of cord measuring 4 x (12 x foot length), with additional cords pre-cut to form the diagonals, so that edges and corners of the square could be aligned at right angles. The decision to use foot length to normalise dimensions of the performance area to participant was influenced by the link segment approach to anthropometric calculation in biomechanics (Winter, 2009, p. 82).

Participants were familiarised with the first ramp of exercise protocol (Sagittal Plane: Forwards-Backwards) by DVD and a researcher provided coaching in exercise actions. Participants were instructed on exercise ramping, according to the approach reported by Gunnarrson et al., (Gunnarsson & Bangsbo, 2012), from low- (30 s) through medium- (20 s) to high-intensity (10 s) during 60s dynamic phases, and use of audio-visual cues on the DVD to initiate change of exercise intensity. Participants were instructed to train twice weekly allowing a minimum of 48 hours between sessions. A training diary was provided to record session dates and adverse advents or muscoloskeletal (MSK) issues, should these arise.

After 4 weeks participants were recalled and familiarised with the second ramp (Coronal Plane: Side to Side) and instructed to train using a DVD as before. This procedure was repeated after 4 further weeks training, when the final ramp (Transverse Plane: Turning) was introduced. All participants had HR and motion characteristics (GPS) recorded during their first and last training sessions.

Table 10 Schedule of Exercise

Weeks 1 - 4	Weeks 5 - 8	Weeks 9 - 12
Sagittal Grid 13.5 min	Coronal Grid 13.5 min	Transverse Grid 13.5 min
Direction of movement emphasis (cardinal plane of loading)		
Forward - Backwards	Side to Side	Turning
60 s periodised 30-20-10 s 4 repetitions 30 s Walk 20 s Jog intermediate speed 10 s Sprint fast	60 s periodised 30-20-10 s 4 repetitions 30 s Side step 20 s Side cut 10 s Side bound	60 s periodised 30-20-10 s 4 repetitions 30 s Walk turn 20 s Run turn 10 s Jump turn
Periodised with elements 1 - 5 below:		
1. Mobilisation: hip, knee ankle, torso		
60 s Sagittal emphasis	60 s Coronal emphasis	60 s Transverse emphasis
2. Balance challenge		
60 s balance x 2 repetitions Unilateral stance sagittal plane hip flexion & extension Repetition with choreographic variation 30 s	60 s balance x 2 repetitions Unilateral stance coronal plane hip ab- & ad-duction Repetition with choreographic variation 30 s	60 s balance x 2 repetitions Unilateral & bilateral stance transverse plane balance Repetition with choreographic variation 30 s
3. High-intensity actions		
30 s x 2 repetitions Jump phase Sagittal plane emphasis 60 s x 1 repetition Fast- forwards- backwards actions	30 s x 2 repetitions Jump phase Coronal plane emphasis 60 s x 1 repetition Fast- side to side actions	30 s x 2 repetitions Jump phase Rotation incorporated 60 s x 1 repetition Fast- COD and turning actions
4. Dance phase 1: 30 s multiplanar dynamic movements 4. Dance phase 2: 150 s multiplanar dynamic movements		
5. Closing phase 60 s		
Sagittal plane Myofascial extensibility	Coronal plane Myofascial extensibility	Transverse plane Myofascial extensibility

Note. COD: change of direction

Sagittal movement: Ramp 1 | Coronal movement: Ramp 2 | Rotational movement: Ramp 3

Figure 8 Exercise Actions During Brief Diverse-Movement HIIT

5.2.11 Locomotor profile and exercise heart rate

Training took place in two locations (Castle Cary, Somerset UK; Streatham Campus, University of Exeter UK) to provide consistency for GPS data acquisition. Additional GPS and HR data were acquired for training sessions attended at these locations on a voluntary basis, depending on participant availability.

All participants attended a minimum of four supervised training sessions: at baseline, and after 4, 8 and 12 weeks' training, during which participants' locomotor characteristics and exercise heart rate (HR) were assessed, using a HR belt and GPS unit, fitted as described in General Methods, 3.5.1. Data were downloaded using manufacturer software (General Methods, 3.5.2), and analysed for the first training session (ramp 1 Sagittal Grid) to provide summary information about locomotor characteristics during initial exposure to diverse exercise.

5.2.12 Perception of effort

Perception of effort was recorded after the first training session at baseline and for supervised session after 4, 8 and 12 weeks, using a visual scale (see General Methods VAS 3.6.2).

5.2.13 Statistical approach

Data were tested for normality (Shapiro-Wilk test) and for data that were normally distributed, paired, two-tailed T-tests were performed using a statistical package (SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.), to compare results before and after 12 weeks diverse exercise. Significance was set at $p < 0.05$.

5.3 Results

5.3.1 Compliance and persistence

Of 11 women recruited to the study, 8 completed 12 weeks training; in these participants, compliance with training sessions was high (99.5%), according to the intervention schedule of 24 training sessions, twice weekly. Amongst those who did not complete the study, one was lost immediately after baseline training, as she no longer met inclusion criteria (attempting to conceive), and 2 participants were lost to non-study related incidents (hip injury; concussion). In these 2 participants, session attendance up to time of incident (38%, and 63% of training schedule, respectively) was 100%.

One participant did not tolerate non-sagittal training (ramps 2 and 3, Coronal and Transverse Grid) citing a pre-existing musculoskeletal (MSK) issue (right knee pain) and continued training for 24 sessions with ramp 1 (Sagittal Grid), which was well tolerated.

5.3.2 Indicators of metabolic and cardiorespiratory status

No statistically significant change was observed in body composition, anthropometrics and fasted glucose after 12 weeks, however there was a slight tendency for resting HR to be reduced (p = 0.086) (Table 11).

Table 11 Participant Characteristics Pre- and Post- 12 weeks (Mean ±SD)

	Diverse Exercise Group (n = 8)		
Characteristic	0 w	12 w	*P*
Age y	47 ±6.0	48 ±6	> 0.999
Height m	1.66 ±0.07	1.66 ±0.07	> 0.999
Weight kg	62.9 ±11.0	63.2 ±10.8	0.399
BMI kg.m^2	22.9 ±1.1	22.9 ±3.2	0.972
Resting HR bpm	76 ±7	73 ±4	0.086
Systolic BP mmHg	131 ±10	127 ±6	0.491
Diastolic BP mmHg	86 ±11	90 ±6	0.108
MAP mmHg	101 ±10	103 ±5	0.340
Fasted Glucose mmol.L^{-1} ⌂	4.4 ±0.3	4.5 ±0.5	0.456

BMI: Body Mass Index; HR: Heart Rate; BP: Blood Pressure; MAP: Mean Arterial Pressure
⌂ n = 7 accessible to venipuncture pre- and post- 12 weeks.

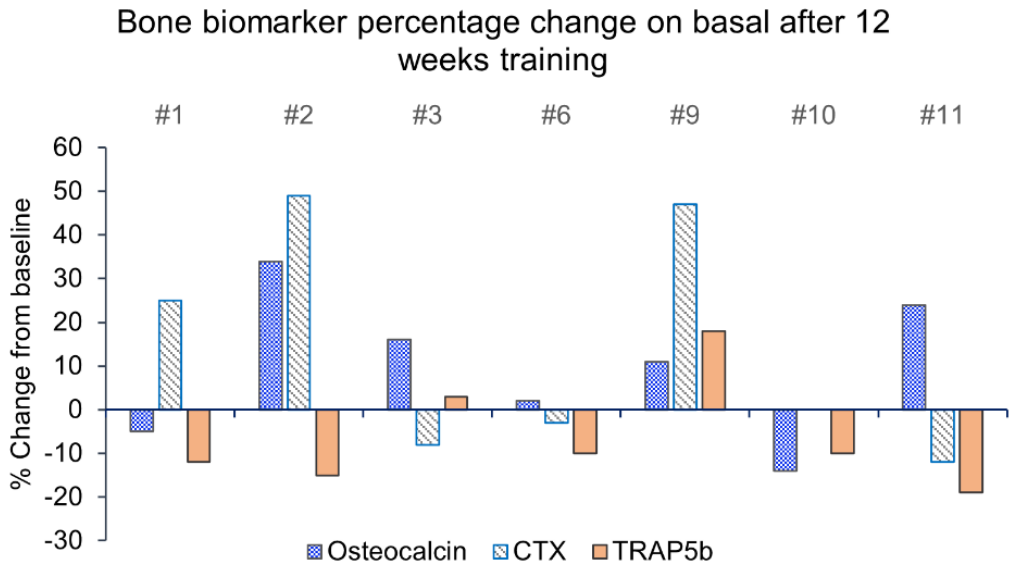
5.3.3 Biomarkers of bone metabolism at rest

One participant who completed the intervention was inaccessible to venipuncture; in participants who provided serum samples (n = 7) there was no significant change in serum OC (16.63 ±8.00 vs 18.14 ±8.36 ng.ml^{-1}, p = 0.119),

CTX-1 (0.42 ±0.31 vs 0.45 ±0.29 ng.ml^{-1}, p = 0.254) or TRAP5b (4.02 ±1.61 vs 3.69 ±1.37 U.L^{-1}, p = 0.112) comparing biomarkers at rest at baseline and after 12 weeks. During standards preparation for bone formation marker P1NP, the laboratory technician performing the assay noted that abnormal reference values were returned. After taking advice, the decision was taken to exclude assay results for this marker, on the assumption that the kit was defective.

According to questionnaires administered at baseline and on completion of the study (see Appendix A1.1), of participants who completed the intervention (n = 8), two were post-menopausal, one of whom was using hormone replacement therapy (HRT) (since 3/12 months) (see #9, Figure 9) and the other was a non-HRT user (see #6, Figure 9), and one participant transitioned into menopause (confirmed by medical blood test) during the intervention.

Percentage change on biomarker value at baseline was calculated to visualise magnitude of individual responses to the intervention (Figure 9).



Note.#6 Postmenopausal Non-HRT user; #9 Postmenopausal HRT user.

Figure 9 Percentage Change in Bone Biomarkers After 12 weeks

5.3.4 Calcaneal stiffness

Body weight and calcaneal BUA were positively correlated in participants at baseline in both the left (r = 0.697, p = 0.017) and right (r = 0.622, p = 0.041) heel (Figure 10), no relationship was observed between body weight and

calcaneal SOS and right SI ($p > 0.05$), however there was a trend for left SI to be positively correlated ($r = 0.568$, $p = 0.068$).

There were no statistically significant differences in left and right calcaneal QUS parameters (SI, BUA, SOS), nor absolute difference between left and right SI, compared with baseline, in those who completed the intervention ($p > 0.05$). Analysis was based on data provided by participants ($n = 7$) at 12 weeks, one participant was unable to attend on the day the ultrasound device was available.

As percentage change on basal, left (L) SI increased by 17.0 ±5.5%, L BUA by 13.9 ±2.7% and L SOS by 1.8 ±0.5% and for the right (R) calcaneus corresponding changes were R SI: -1.7 ±5.6%, R BUA: -0.3 ±4.1% and R SOS: -0.3 ±0.4% (Figure 11).

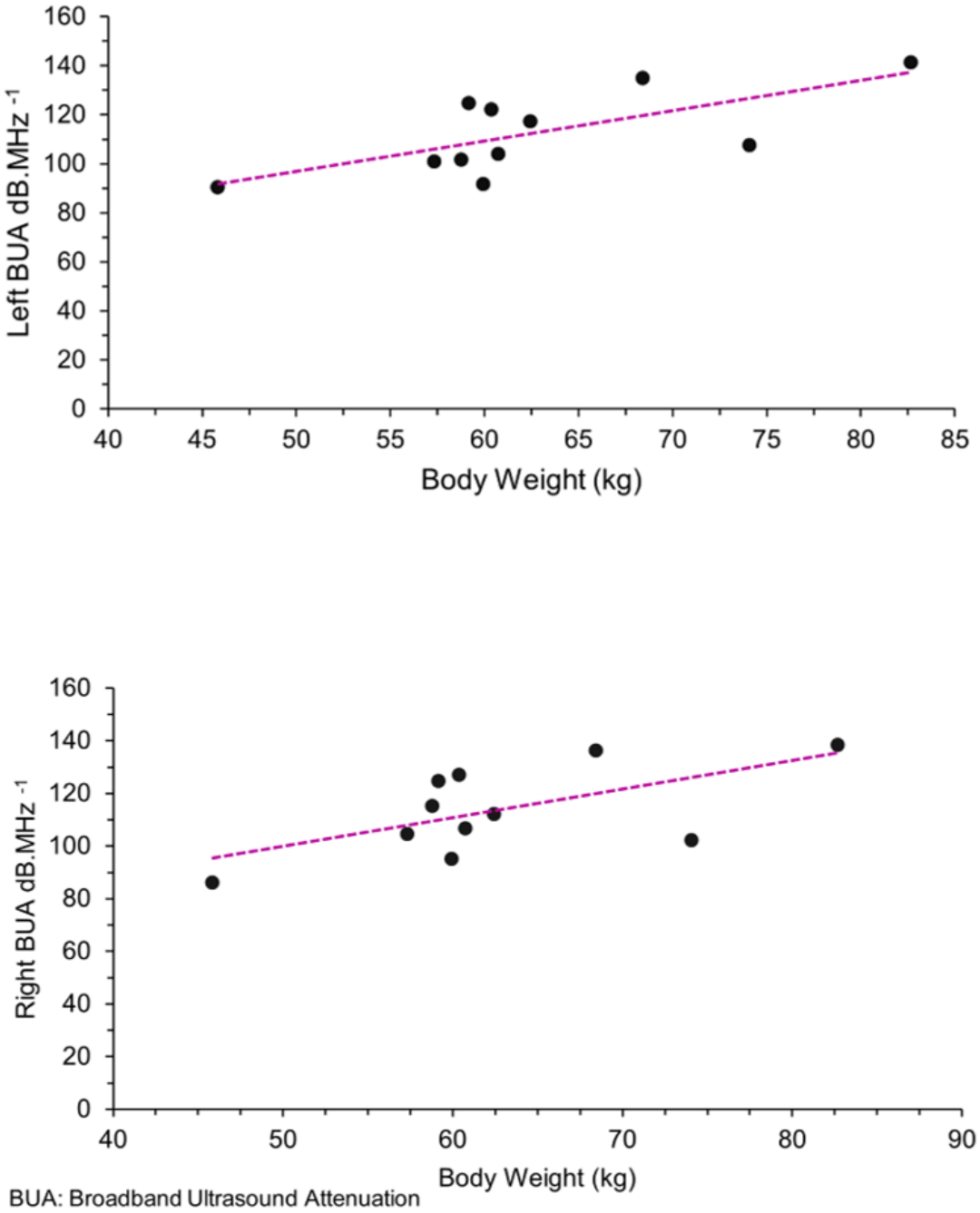
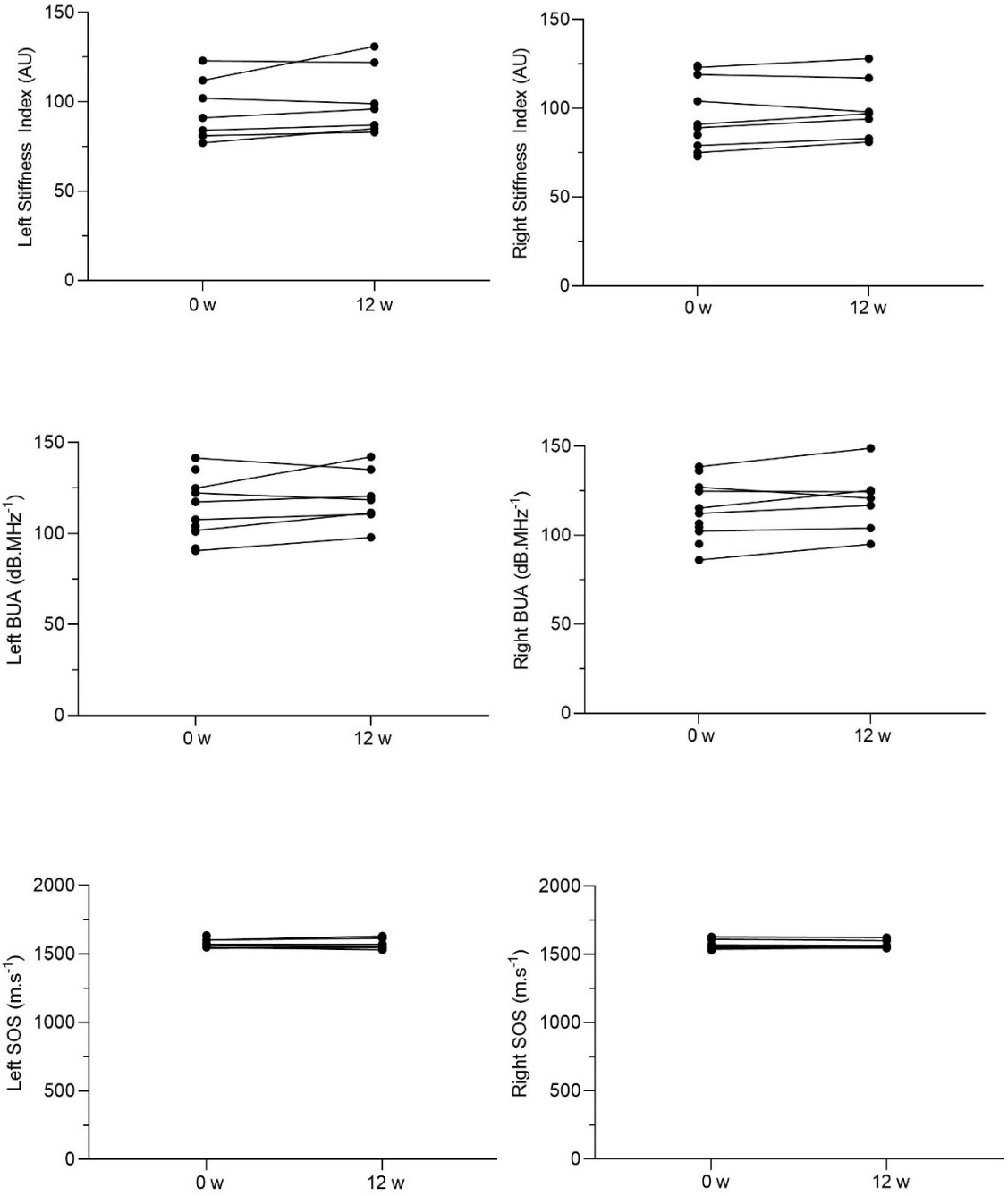


Figure 10 Calcaneal BUA And Body Weight At Baseline



BUA: Broadband Ultrasound Attenuation; SOS: Speed of Sound.

Figure 11 Calcaneal Ultrasound Pre- and Post- 12 Weeks

5.3.5 Physiological demands of acute diverse HIIT

Heart Rate during exercise

During baseline training with the Sagittal Grid (ramp 1) mean exercise HR was 84.0 ±6.9% of HR maximum predicted by age (220 – age [y]) (Table 12) and peak HR was 5.6 ±8.3% above maximum predicted by age.

Locomotor profile

Distance covered, mean and peak speed during exercise are summarised below (Table 12); accelerometer data indicated 101 ±75 impacts were recorded > 4 g during initial training.

Visual inspection of accelerometer profile and locomotor characteristics, using the time stamp provided in manufacturer software, showed agreement between profile of consolidated accelerations and the progressive ramp of intensity during the first 60 s of the exercise bout (Walk-Shuttle-Run for 30-20-10 s) (Figure 12).

Perception of effort

Participants rated demands of diverse HIIT as 64 ±12 for overall challenge ($VAS_{CHALLENGE}$), 63 ±16 for demand on legs (VAS_{LEGS}) and 70 ±5 for demand on breathing ($VAS_{BREATHING}$). One-way Analysis of Variance showed no significant difference between response categories ($p = 0.283$).

Using separate correlations to examine the relationship between VAS score for each category and total distance covered during exercise, and total number of impacts > 4 g, no relationship was observed between perception of effort and locomotor data ($p > 0.05$).

Table 12 Heart Rate and Locomotor Profile During Baseline Diverse HIIT

Summary	Exercise Heart Rate (HR)				Locomotor Characteristics				
(*n* = 11)	HR_{MAX} bpm	HR_{MEAN}% $HR_{MAXPRED}$	HR_{MEAN} bpm	HR_{MIN} bpm	Tot Distance m	$Speed_{MAX}$ $km.h^{-1}$	$Speed_{MEAN}$ $km.h^{-1}$	$Impact_{MAX}$ g	Impacts >4g count
Mean ±SD	184 ±17	84.0 ±6.9	146 ±14	77 ±11	515.8 ±148	11.7 ±2.7	1.5 ±0.4	7.5 ±1.7	101 ±75

HR_{MEAN} % $HR_{MAXPRED}$ %: Mean HR during exercise as a percentage of age predicted maximum (220 – age [y])

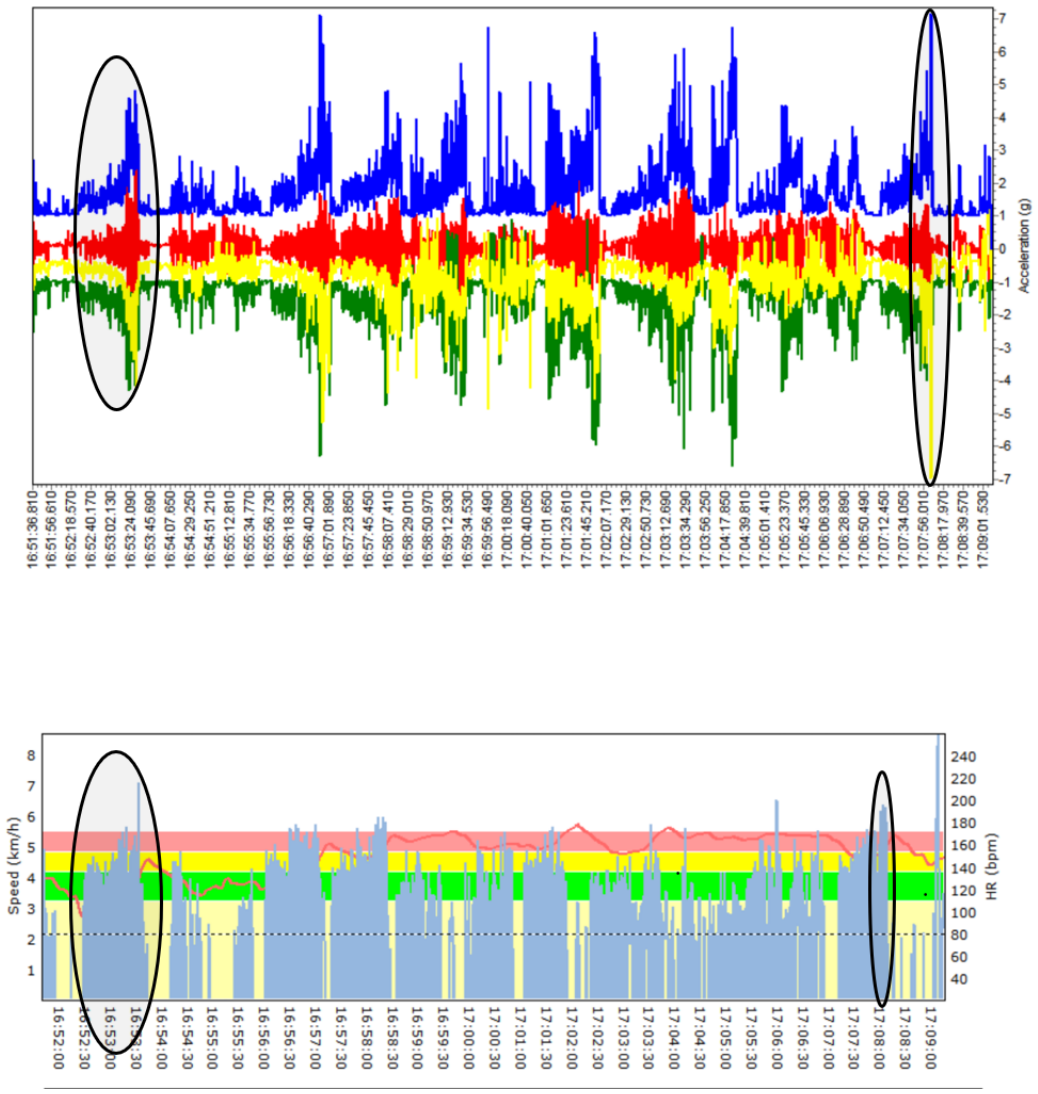


Figure 12 Exemplar of Acceleration Profile During Diverse Exercise

5.3.6 Flamingo balance test

A tendency for fewer restabilisations during the balance test was observed after 12 weeks (9 ±9 versus 4 ±3, $p = 0.064$) (Figure 13).

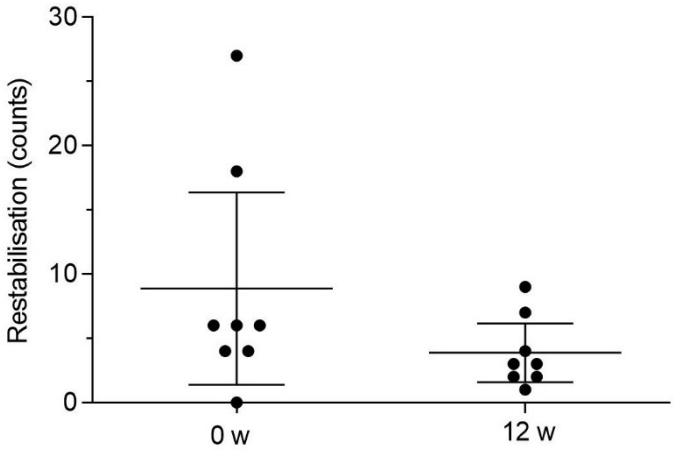


Figure 13 Flamingo Balance Pre- and Post- 12 weeks (Mean ±SD)

5.4 Discussion

The primary aim of this study in sedentary females was to examine feasibility and acceptability of brief exercise in an audiovisual format, which could be streamed to enable autonomous training. Evidence from training logs and self-report showed compliance and persistence with diverse HIIT prescribed as brief (<15 min) training bouts was high, and format of prescription was acceptable. In eight women who completed the 12 week study, 99.5% compliance with training was recorded, and there were no adverse events or musculoskeletal (MSK) issues associated with exercise. Compliance in two participants, who withdrew for non-study related reasons, was 100% up to point of withdrawal; another participant left the study before initiating training, as she no longer met inclusion criteria.

Although there was no evidence of an effect of the intervention on calcaneal stiffness and background bone biomarkers, locomotor profile during baseline training demonstrated 101 ±75 impacts of accelerations above 4 g, a level characterised as providing an osteogenic stimulus (Jämsä, Ahola, & Korpelainen, 2011). Moreover, mean exercise heart rate (HR) was 84.0 ±6.9% of peak HR predicted by age, exceeding a proposed threshold for HIIT (Ito, 2019). Therefore, whilst diverse HIIT as prescribed was not associated with an observed osteogenic effect after chronic training, evidence suggests initial exposure imposed an acute physiological demand on inactive females and elicited accelerations above the threshold demonstrated to be osteogenic in this population.

A novel dimension of the protocol was that dynamic actions were choreographed and prescribed in escalating phases of intensity, from 30 s ‘low‘, through 20 s ‘intermediate‘ to 10 s ‘high’, which in the first and last 60 s phases of each exercise bout comprised 30 s walking, followed by 20 s jogging, and ended in 10 s sprinting at ‘all out’ intensity. Acceleration profile during initial training in the basal state showed that peak acceleration (7.5 ±1.7 g) occurred during the final 10 s of these 60 s ‘walk-shuttle-run’ phases. In vivo findings in humans have shown running and jogging on a level surface can elicit principal compressive strains of ~2000 microstrains (Burr et al., 1996), mechanisitically proposed to act as an anabolic stimulus to bone mediated by the basic multicellular unit (BMU), which acts as a bridge between cellular responses and

morphological bone adaptation (Robling, Castillo, & Turner, 2006). Moreover, longitudinal examination of acceleration profile during daily PA in premenopausal females (35 – 40 y) has shown exposure to accelerations above 4 g is positively associated with changes in femoral and hip BMD and geometry (Ahola et al., 2010), providing a putative threshold for bone adaptation for accelerations derived from daily PA, in accordance with the mechanostat principal (Frost, 1987). Furthermore, an important aspect of findings reported by Ahola and colleagues is that both intensity, characterised by magnitude of acceleration, and number of repetitions (i.e. frequency in daily PA) were determinants of osteogenic potential for activities assessed by daily impact score (DIS) (Ahola et al., 2010). Applying this rationale for DIS to locomotor profile during diverse HIIT at baseline, which showed there were an average of 101 ±75 impacts surpassing the 4 g threshold during vigorous epochs, absence of an effect on calcaneal bone could be attributed to frequency, rather than intensity, characteristics. It may therefore be hypothesised that volume of high-intensity activities, rather than thresholds surpassed during execution, was insufficient to elicit a quantifiable osteogenic effect. Therefore, according to the proposal of 'exercise as medicine' (Pedersen & Saltin, 2015), results suggest a requirement to increase dosage in future delivery of diverse HIIT, to assess whether this elicits an observed anabolic effect on bone. Whilst this could be achieved by extending bout duration and/or increasing weekly training frequency, it could also be effected by modifying the protocol, to lengthen bouts of actions demonstrated in locomotor profile to elicit high-intensity accelerations.

During initial training, 10 s 'all out' phases, which comprised progressive sprinting or bounding and vertical jumping or hopping, and where highest impacts were observed, were followed by either 30 s of progressive actions at low intensity, or 30 – 120 s static mobility and balancing. Overall, durational ratio of high-intensity : low-intensity : intermediate-intensity : static balance/mobility was 1.0 : 2.3 : 1.5 : 3.6 (s). It is possible that this ratio was inadequate to deliver high-intensity actions at sufficient density to elicit osteogenesis, which supports a hypothesis of under-dosing. For example, at the start of exercise, the initial 30 – 20 – 10 s phase, corresponding to walk – jog – sprint, was followed by 120 s recovery, and examination of locomotor profile showed negligible impact and low (1 – 1.5g) accelerations during this phase. Although choreography was informed by evidence that insertion of deliberate

rest periods reduces accommodation to the mechanical signal delivered by exercise (Meakin et al., 2014), and can enhance the osteogenic potential of a regimen (Srinivasan, Weimer, Agans, Bain, & Gross, 2002), results suggest the refractory period of recovery may have been too long. Hypothetically, high-intensity signals could be amplified and exposure frequency increased by reducing duration of low-intensity and recovery in future implementation, and inserting additional epochs of high-intensity actions.

Whilst modifying protocol content does not address limitations arising from overall duration of the present intervention, regional gains in BMD, from team sports prescribed at low-duration, provide some evidence of an osteogenic effect for exercise below the 4 – 6 months timecourse proposed for bone adaptation (Clark, 2008). In a study in premenopausal women that compared running and football, a team sport which involves exercise actions used in the present protocol, such as lateral cutting, sagittal shuttle runs, hopping and jumping, an anabolic response in tibial BMD, quantified by peripheral quantitative ultrasound (pQCT), was reported after 14 weeks, and was greater after football than running (Helge et al., 2010). Whilst overall duration of the study by Helge and colleagues was similar to the present (14 weeks versus 12 weeks), volume of weekly exposure was greater, at 1. 8 ±0.3 h for football and 1.9 ±0.3 h for running, compared with ~ 0.5 h for diverse HIIT, and for the same duration (14 weeks) of increased training volume, tibial volumetric BMD was also reported to increase in adolescent male footballers (Varley, Hughes, Greeves, Fraser, & Sale, 2017). Both these, the present investigation, quantified exercise effects at bone sites close to the source of impacts, where attenuation of torsional and bending moments arising from impacts is low (Yang et al., 2014), either by using pQCT at the tibia (Helge et al., 2010; Varley et al., 2017), or calcaneal ultrasound in diverse HIIT participants. Evidence of tibial adaptation from football training after 12 – 14 weeks, which contrasts with no effect of 12 weeks diverse HIIT on calcaneal bone, tends to support an interpretation of insufficient volume of high-magnitude impacts per bout to promote an osteotropic effect, rather than necessarily overall duration of the present intervention. Moreover, a significant positive correlation between left and right calcaneal BUA and body weight, as observed in participants at baseline, tends to suggest directionality in the relationship between body weight and mechanical effects of longterm loading from PA, in

agreement with population based evidence in premenopausal women (Adami et al., 2004) and children and adolescents (Xu, Guo, Gong, Xu, & Bai, 2014). Future interventions could explore potential interactions between body weight, acceleration profile and calcaneal bone response to diverse HIIT. Whilst no relationship between body mass and peak magnitude impact was observed here during initial diverse HIIT exposure, it is possible that basal body weight could exert a positive effect on calcaneal bone during longer-term training, by increasing impact intensity, as a result of incurring higher ground reaction forces during landings. Alternatively, it could be hypothesised that the requirement to overcome inertia during whole-body COD at the edge of the exercise grid could give rise to a negative relationship between body weight and high-intensity acceleration characteristics. It would be of interest to examine this proposal, as it suggests reducing exercise area could be a disincentive to performing high-intensity actions, affecting potential acceptability of diverse HIIT in an overweight inactive population.

It is also possible that high-intensity actions observed during initial training could have induced adaptation in bone after 24 sessions, however anabolic effects were not detectable at the level of resolution provided by QUS. For instance, in murine models, tibial bone after short-term treadmill exercise has been shown to exhibit increased tensile strength and reduced microdamage, without evidence of cross-sectional changes in bone geometry (Kohn, Sahar, Wallace, Golcuk, & Morris, 2009). Furthermore, as trabecular architecture, particularly density and prevailing alignment of spiculae, degree of matrix mineralisation, and collagen properties, are also determinants of bone strength (Fonseca, Moreira-Gonçalves, Coriolano, & Duarte, 2014), tissue-level adaptation in bone can occur in the absence of meaningful changes in BMD. It is possible that mechanical adaptation in calcaneal bone in response to high-intensity actions was undetected by calcaneal ultrasound and therefore cannot be ruled out. Although calcaneal QUS indices have been positively correlated with DXA, and therefore a role in predicting fracture risk is supported (Hans & Baim, 2017), precision errors for QUS, both short-term and longer-term, are higher than for DXA, which is the gold standard (Njeh et al., 2000), therefore a true change in bone may not have been detected due to measurement imprecision. However, bone biomarker results, which did not indicate an effect of diverse HIIT on background bone formation-resorption dynamics, support the

hypothesis that the stimulus was not sufficient to exert constitutive effects on bone metabolism.

Whilst low numbers of participants providing baseline and 12 weeks blood samples (n = 7) limits generalisation of results, biomarker data did provide single-case insight into bone metabolic responses to diverse HIIT in postmenopausal participants who completed the study, one of whom was an HRT user (HRTU) whereas the other was not (HRTN). After 12 weeks, percentage change on basal bone biomarkers in HRTU was 11% for OC (marker of formation), and 47% for CTX-1 and 18% TRAP5b (markers of bone resorption), whereas in HRTN corresponding values were 2% (OC), -3% (CTX-1) and -10% (TRAP5b), respectively. On a case by case basis, results suggest there was capacity for lability in bone biomarkers dynamics in the individual taking exogenous oestrogen, whereas amplitude of change in basal background biomarkers after 12 weeks was low in HRTN. As gonadal steroid hormone status was not formally examined, and participant numbers were low, it is not possible to quantify the relationship between menopausal status and bone metabolic responses to diverse HIIT. In any case, the effect of HRT on postmenopausal responses to exercise is controversial. For example, no influence of HRT on regional and whole-body gain in lean mass, and reduction in lower limb and whole-body percentage fat mass, were reported after long-term (1 y) weight-bearing aerobic exercise in women of similar status (early premenopause) to those in the present study (Figueroa et al., 2003), whereas more recent meta-analytic evidence has found an additive effect of HRT on bone anabolic responses to exercise postmenopause (Zhao, Xu, & Zhao, 2015). In agreement with the latter proposal, regional BMD response to 1 y aerobic and weight-lifting exercise was more extensive in HRTU than HRTN (Going et al., 2003), and HRT supplementation shown to improve vertical jump height and 20 m sprint time compared with HRTN in response to 1 y impact training in women aged 50 – 57 (Taaffe et al., 2005). Accordingly, it may be speculated that MSK responses to diverse HIIT could be mitigated under conditions of postmenopausal hypooestrogenism, if capacity for high-threshold accelerations and high-magnitude impacts was reduced, as a consequence of lower muscular performance (Sirola & Rikkonen, 2005).

5.5 Limitations

Whilst the primary aim of demonstrating acceptability of diverse HIIT was achieved, a limitation of the present study was the decision to accept women based on age (35 – 55 y), rather than menopausal status. This was helpful, in that it enabled acceptability of diverse HIIT to be examined in women at different life stages but introduced potential confounding effects from differences in gonadal hormones status. In future implementation of diverse HIIT, oestrogen status should either be controlled for, or menopausal condition (e.g. pre-, peri- or post-) standardised, to eliminate the influence of gonadal hormone lability and distinct oestrogenic states in evaluating acute and chronic effects of the protocol on bone. This could be addressed by narrowing inclusion criteria for age. Another limitation is that ramping of exercise was undertaken by cardinal direction of loading, i.e. dominant plane of movement during exercise. Therefore, the effect of intensifying the exercise stimulus by increasing the duration of high effort bouts, or including these phases more frequently and reducing recovery, was not addressed, and should be in future studies. As a consequence of this approach to ramping, basal acceleration profile and exercise heart rate were not compared with data after 12 weeks, due to differences between the protocol prescribed in the first and final phases of the study. Administering a specific diverse HIIT protocol at baseline and exit, as a test-retest method for quantifying the effects of chronic training on accelerations and exercise intensity, could overcome this limitation. Finally, whilst motion sensor units provided an impact score derived from consolidation of triaxial accelerations, it was not possible to characterise duration of accelerations according to direction, nor could the unit be used to quantify exercise indoors, as generation of acceleration profile was dependent on an accessible GPS signal. In future, accelerometers with capacity for orthogonal discrimination could enable diverse HIIT to be examined in an indoor setting, and yield direction-specific insight into potential relationships between vertical accelerations during exercise, and responses in bone.

5.6 Conclusion

This study found high acceptability and compliance with brief, self-regulated diverse HIIT in sedentary females. Whilst results showed exercise actions elicited high-magnitude accelerations and elevated heart rate, volume of

high-intensity activities prescribed in the protocol did not elicit an observed osteogenic effect in these female participants of mixed menopausal status. The resulting differences in gonadal hormone status confounded interpretation of bone biomarker findings. Future studies, which address limitations of prescription identified in the present investigation, could provide insight into chronic effects of diverse HIIT and the relationship between bone biomarker responses and acceleration profile, particularly volume of exposure to accelerations in the vertical direction.

6 Characterising Locomotor Profile and Acute Physiological Demands of Diverse Movement-HIIT (DM-HIIT) In Sedentary Women and Female Dancers

6.1 Introduction

As a form of structured exercise, team sports in a recreational setting have been demonstrated to enhance health outcomes. Beneficial effects on systolic and diastolic blood pressure, resting heart rate, lipid regulation and fat mass, and greater leg power were reported in a meta-analysis of the health benefits for recreational football (Milanović et al., 2019). In young (~24 y) sedentary females, 12 weeks of twice-weekly handball significantly increased muscle mass by 2.1% and proximal femoral BMD by 0.8% in the women who participated in team sports, compared with non-exercising controls (Hornstrup et al., 2018).

There is also evidence of osteogenic effects for longer term team sports participation from cross-sectional studies in athlete populations (Hinrichs, Chae, Lehmann, Allolio, & Platen, 2010; Mudd et al., 2007) however, the osteogenic stimulus provided by exercise under these conditions is not universal. For example in a cross-sectional study, in rank order, greater total BMD has been observed in female volleyball players, followed by footballers, field hockey players and swimmers at collegiate level (Bellver et al., 2019). This suggests that bone anabolic effects of exercise are mediated by interactions between sports-specific factors, such as loading environment, type of mechanical strain elicited by participation (e.g. multidirectional versus monotonic) and phenotypic characteristics of participants within sporting domains.

Mechanistically, osteogenic effects of team sports have been attributed to diverse (i.e. non-uniform) skeletal loading incurred during high-intensity, participatory actions and unpredictable mechanical strains arising from landing impacts (Krustrup, Helge, et al., 2018; Póvoas et al., 2017; Zouhal et al., 2020). Measurement of tibial bone adaptation, using peripheral quantitative computed tomography (pQCT), has shown that impact is a critical component of skeletal strains during team sports participation. In adolescent male footballers, trabecular and cortical density at the tibia increased by 4% and 14%, respectively, alongside 14% increase in strength-strain index after 12 weeks

ramping of training load (Varley et al., 2017). In previously inactive females, significant bilateral increases in tibial volumetric BMD were observed after 14 weeks football as small-sided games (SSG) (Helge et al., 2010). As ground reaction forces are only minimally attenuated at the tibia (Gross & Nelson, 1988), and are predominantly axial (i.e. compressive) at this site during gait (Wehner, Claes, & Simon, 2009), pQCT results suggest that axial loading from landing impacts are significant contributors to localised osteogenic responses from team sports participation (Helge et al., 2010; Varley et al., 2017). Hypothetically, prescribing consecutive flighted actions at high density could increase the osteogenic potential of exercise, by enhancing frequency of landing impacts and associated axial loading of the skeleton.

Alongside direct mechanical forces from ground impact, muscular contractions during exercise impart mechanobiological strains, which lead to skeletal adaptation in response to overload (Burr, 1997). The osteogenic effects reported for longterm team sports (Hagman et al., 2018; Lozano-Berges et al., 2018) may partly be attributable to contractile forces exerting direct mechanical effects on bone during exercise (Baker et al., 2013), as well as muscle-bone crosstalk, mediated by myokines such as irisin (Colaianni, Mongelli, Colucci, Cinti, & Grano, 2016). According to a proposed musculoskeletal pleiotropy, whereby morphological changes in muscle and bone are fated to evolve in parallel (Cianferotti & Brandi, 2014), there is a rationale for exercise which solicits intense, consecutive muscular efforts producing co-adaptive effects in bone. Irisin, a muscle secretome produced in response to exercise, has been identified as a candidate effector of muscle-bone cross-talk (Liu, 2015), shown to increase osteoblastic bone formation in an animal model (Colaianni et al., 2015) and proposed to have a protective effect on bone health (Colaianni et al., 2016). According to the proposal of bone-muscle cross-talk, ‘muscle work’ also provides ‘bone work’ mediated by myokine activity, alongside paradigmatic biomechanical effects of skeletal loading incurred during exercise.

In an applied context, whole-body take-off and landing actions, as required during change of direction (COD), lateral cutting or ball heading, not only elicit episodes of axial compression on bone through vertical impacts, but also place high concentric and eccentric demands on polyarticular muscles that influence angular motion at the hip, knee and ankle (Yu, Lin, & Garrett, 2006).

There is evidence that ~70% of bending moments on bone during lower limb gait actions are offset by tensile forces applied by muscles (Lu, Taylor, O'Connor, & Walker, 1997), which further supports the proposal of functional coupling between muscle and bone during musculoskeletal loading (Cianferotti & Brandi, 2014; Schoenau, 2005). It follows that exercise actions demonstrated to upregulate metabolic demands on muscle, as shown for frequent COD even at low velocities (Hatamoto et al., 2014), could exert an osteogenic effect through bone-muscle interactions. Therefore, it may be hypothesised that emphasising multidirectional accelerations and decelerations during a bout of exercise, by deliberate prescription of consecutive COD, jumping and cutting, could provide an osteogenic effect from tensile muscle forces, coupled to bending moments generated by COD actions on bone.

After observing osteogenic effects of small-sided football games at reduced duration (see Chapter 4, sections 4.3.4 and 4.3.7), we developed a new exercise programme, based on the principles of HIIT, utilising multidirectional actions and change of direction (COD) at high density, as observed during intense phases of team sports (Bangsbo, Mohr, & Krustrup, 2006; Harper et al., 2019). High compliance and acceptability with brief, diverse exercise in this format was shown in pre- peri- and postmenopausal females (see Chapter 5, section 5.2.10), and the protocol was modified to include more vertical jumping and progressive, lateral actions. Two design features from the regimen assayed in the feasibility study were retained: constraining exercise area (3 m x 3 m) and ramping exercise intensity according to the 10 – 20 – 30 s approach (Gunnarsson & Bangsbo, 2012).

Although there is a precedent for using accelerations derived from locomotor profile to describe osteogenic features of physical activity (PA) (Jämsä et al., 2011; Rowlands et al., 2019), interventional prescription of accelerations in a structured HIIT scenario has not been undertaken previously. Furthermore, algorithmic consolidation of triaxial accelerometric data into a scalar resultant has been adopted, for example, in population studies (Karantonis, Narayanan, Mathie, Lovell, & Celler, 2006) and characterisation of player load in team sports (Dalen, Jørgen, Gertjan, Geir Havard, & Ulrik, 2016). However, such an approach does not enable the relationship between direction (orthogonal) and magnitude (threshold) within acceleration profile to be explicitly

characterised, although these are demonstrated to be critical determinants of adaptation in bone (Turner, 1998). Therefore, a further novel aspect of this study is the proposal to quantify acceleration characteristics in each orthogonal separately, using thresholds for durations of both linear and angular accelerations, to provide direction-specific insight into locomotor profile during exercise.

In summary, this study aims to characterise acceleration profile and physiological demands of a novel iteration of diverse movement HIIT (DM-HIIT) in two female populations, recruited to participate in a single bout of exercise, in order to evaluate osteogenic potential and intensity of the exercise stimulus.

6.2 Methods

6.2.1 Study design

This was a cross-sectional examination of locomotor profile and acute physiological responses to a single bout of diverse-movement HIIT (DM-HIIT) exercise in two female populations. Participants were recruited from a performance athlete cohort (*n* = 19 ballet dancers in the final year of pre-professional training) according to convenience sampling, and 15 sedentary premenopausal females were recruited to provide a comparator group, representing a non-athlete female population. Randomisation was not undertaken: all females within the dancers' year group were invited to participate in the study if they met inclusion criteria, and sedentary females were recruited to a single bout of DM-HIIT, unless excluded during participant pre-screening. As the primary research aim was to characterise responses to a novel protocol, effect sizes have not been reported for diverse HIIT and power calculations were not undertaken. Moreover, as dancers are a specialised athlete population, inferences drawn from the cohort's responses would be expected to be of limited generalisability in estimating whole population effects for a single bout of training.

6.2.2 Participants

Recruitment Sedentary women (*n* = 15) from the south west of England were recruited by poster campaign and leaflet drop. Written, informed consent was obtained from those women who met inclusion criteria:

- aged 18 or older
- menstruating regularly
- not pregnant
- not participating in more than 150 minutes moderate intensity exercise per week
- generally healthy
- no known contraindications to participating in an acute bout of varied intensity exercise

All procedures involved in participation, and the aims of the study, were explained by the investigator, and participants given the opportunity to raise concerns or ask questions. The study was approved by the Sport and Health Science ethics committee at the University of Exeter (see Appendix A2.1).

Female dancers (*n* = 19) in their final year of full-time pre-professional training (Central School of Ballet, London), who met the following inclusion criteria were also recruited:

- menstruating regularly
- not pregnant
- generally healthy
- no known contraindications to participating in an acute bout of varied intensity exercise

Female dancers gave written informed consent to participate in an interventional study (described in Chapter 8), which was separately approved by the local ethics committee (see Appendix A2.4). Baseline data in female dancers from the 12 week training study were compared with sedentary females, who undertook a single bout of the same diverse HIIT protocol.

6.2.3 Schedule for data collection

Participant data were collected during a single visit to a training location (sedentary females) and during baseline data gathering in female dancers (see Chapter 8, 8.2.4).

6.2.4 Anthropometric data collection

Participant weight (kg) and height (m) were measured (see General Methods 3.2.1 and 3.2.2) and an average calculated from three consecutive measurements (Table 13).

6.2.5 Heart rate and blood pressure at rest

Heart rate and blood pressure at rest were measured after participants had sat quietly for ten minutes (see General Methods 3.2.5). An average was calculated from three consecutive measurements (Table 13).

6.2.6 Medical and lifestyle questionnaires

A questionnaire (see Appendix A1.1) was used to gather information about medical and exercise history, menstruation status and contraceptive use, and consumption of alcohol and tobacco. Weekly calcium intake was assessed by a questionnaire (see Appendix A1.2) validated for evaluation of dietary calcium (Sebring et al., 2007) (Table 13).

Table 13 Summary of Participant Characteristics (Mean ±SD)

	Intervention Group		
Characteristic	**DF (*n* = 19)**	**SF (*n* = 15)**	***p* value**
Age (y)	18.6 ±1.2	35.3 ±6.4	< 0.001
Height (m)	1.66 ±0.07	1.66 ±0.05	0.659
Body weight (kg)	53.10 ±5.62	69.72 ±13.94	< 0.001
BMI ($kg.m^2$)	19.1 ±1.5	25.5 ±5.8	< 0.001
HR (bpm)	82 ±10	72 ±13	0.018
Systolic (mmHg)	105 ±12	114 ±11	0.024
Diastolic (mmHg)	65 ±10	80 ±11	< 0.001
Ca ($mg.d^{-1}$)	1556 ±798	1006 ±339	0.012

DF: Female Dancers; SF: Sedentary Females; BMI: Body Mass Index; HR: Heart Rate.

6.2.7 Quantitative ultrasound of the calcaneus

A quantitative ultrasound scan (GE Lunar Achilles Insight, Bedford, UK) was performed on the left and right calcaneus (See General Methods 3.2.8) as described in General Methods, 3.2.8.

6.2.8 Exercise protocol

Participants were familiarised with DM-HIIT by DVD and a streaming link prior to the training bout, which gave access to a short film that provided an audio-visual demonstration (link provided in A3.2).

Before exercise, participants were fitted with a heart rate monitor (Polar T31, Polar, Finland) and a movement sensor unit (OptimEyeS5, Catapult Sports, Australia) worn in a close-fitting vest, supplied by the manufacturer (see General Methods 3.5.1). Participants were instructed to stand still for 10 s after the unit was switched on and then performed a bout of submaximal exercise (see General Methods 3.7 paragraph 2). After the first capillary lactate sample, participants undertook diverse-movement HIIT (DM-HIIT), which is summarised as a protocol in Table 14.

Each participant exercised within a 3 m x 3 m space marked out as a grid using flat yellow tape adhered to the floor to mark the edges. The grid was aligned to allow unimpaired visual and audio access to the test demonstration and commentary, projected on a screen in front of the exercise area. Immediately after completing DM-HIIT the motion sensor unit was switched off.

Table 14 Diverse-Movement HIIT (DM-HIIT) Schedule

Section Number & Title	Duration (s)	Description
1. Walk Shuttle Run	60	– **30 s walk** alternate foot during front line & back line COD – **20 s jog/shuttle l**and positively (plantar impact) on front line of grid, maintain stiff hip-knee-ankle, accelerate from back line, alternate feet during COD – **10 s run** self-selected 'fastest runing' pace; deliberate braking action on front line & push off from back line of grid
2. Preparation Phase	90	– **4 x Bilateral heel raise** plantar flexion knees extended – **4 x Bilateral heel raise** hip flexion - extension maintaining heel height and knee position: isometric action of lower leg – **External to internal hip rotation** 2 x L-R hip dissociation – **Unilateral hip flexion balance challenge** 1 x L- R – **Lunge forwards & backwards ipsilateral leg** repeat with contralateral leg 1 x L-R – **Sumo frontal plane lunge** 1 x L-R – **Lunge into trunk rotation** 1 x L-R – **Side flexion trunk** 1 x L-R – **Thoracic extension – flexion** 2 x each gesture
3. Walk Shuttle Run	60	– **As Section 1**
4. Giant Steps	25	– **Forward hip flexion** gesture into full knee extension – **Step out land on gesture leg** balance – **Reverse gesture** extending hip – **Step back onto foot of gesture leg** balance – **Repeat** on contralateral leg
5. Cutting Section	60	– **30 s side cutting** steady pace – **20 s side leap** into faster cut: dynamic side bounding – **10 s fast cutting** L⇔R with side bound
6. Giant Steps Sideways	25	– **Flex hip and extend in coronal plane** land on foot of gesture leg: maintain balance & repeat gesture stepping back onto contralateral leg – **Repeat sequence** alternating legs to end of epoch
7. Cross Step and Shuttle	50	– **30 s cross step** to back line – **Run forward** – **Side stepping** to opposite corner – **Repeat cross step** backwards on other diagonal
8. Balance	20	– **One leg balance** – **Extend gesture leg** modified Y balance forward-side-side backwards side-side – **Repeat balance** on contralateral leg
9. Lunges	60	– **30 s forward lunge** reverse & backward lunge from front edge of grid – **20 s jump** into single foot landing **Absorb** stepping contralateral leg together with landing leg **jog back** – **10 s vertical jumps** as many as possible in time epoch
10. Balance-	40	– **As section 8** – **Additional repetition** sustaining balance on 1 leg with heel off floor **(isometric plantar flexion)**
11. Footwork Sideways	30	– **15 s travel laterally edge to edge along the back line** stepping off and onto the forefoot – **15 s repeat sequence** increase speed
12. Balance	15	– **Unilateral balance** flex gesture leg at hip attempt plantar flexion if possible (vertical acceleration over planted foot) – **Repeat** on contralateral leg
13. Footwork Forwards into Balance	30	– **15 s vertical ladder** progressing forwards along one edge of the box – **15 s increased speed** same movement sequence
14. Balance	15	– **As section 12**
15. Walk Shuttle Run	60	– **As section 1**
16. Close Down	40	– **Back Lunge L and R** upper torso rotation in lunge stance – **Standing** trunk side bend – **Standing** upper thoracic trunk extension & flexion

6.2.9 Lactate sampling

A capillary blood sample to measure lactate was obtained (see General Methods 3.4.2) immediately after submaximal exercise and immediately and two minutes after DM-HIIT. Participants were instructed to remain standing whilst samples were taken.

6.2.10 Perception of exercise effort

Rating of exercise effort was undertaken using a visual analogue scale (see General Methods 3.6.2) between obtaining the second and third capillary blood sample. Participants remained standing during rating of effort.

6.2.11 Acceleration profile

Linear and angular acceleration data acquired during submaximal and DM-HIIT were downloaded (Catapult Sprint 5.1.7, Catapult Sports, Australia) and split into two data files, corresponding to submaximal and DM-HIIT, which were then exported from the manufacturer's download environment (.csv file format) to permit further data analysis (see General Methods 3.5.3 and 3.5.4). From examination of raw data, acceleration bands for submaximal and DM-HIIT were characterised as described in Table 15.

Table 15 Acceleration Bands for Submaximal and Diverse-Movement HIIT

	Linear Acceleration g Bands					Angular Acceleration R Bands				
Bout	1-1.5	1.5-2	2-2.5	2.5-3	>3	30-60	60-90	90-120	120-150	>150
DMHIIT	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
SubMax	✓	✓	-	-	-	✓	✓	-	-	-

Note. g Band: denotes acceleration due to gravity 9.8 m.s-2 .R: radians, denotes angular acceleration in degrees.s^{-2}.

6.2.12 Statistical approach

Data were analysed using a statistical software package (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.).

Participant characteristics, calcaneal ultrasound (QUS) data, and heart rate during submaximal exercise and DM-HIIT, were assessed for normality (Shapiro-Wilk normality test) and examined for outliers (box plot inspection) and an independent T-test used to compare between groups for each parameter, where data observed a normal distribution. A Mann-Whitney U test was used for data with a non-Gaussian distribution. Sample size was inadequate for

comparison of questionnaire responses (menstrual status, alcohol and tobacco consumption) using chi-square test for homogeneity and therefore responses were summarised narratively (see Results 6.3.1).

To examine the relationship between participant body mass and calcaneal stiffness, correlations between calcaneal QUS outcomes and BMI were performed with absolute QUS data, and with QUS data normalised to participant body mass.

Three-way analysis of variance (ANOVA) was performed on acceleration data, with ‘group’ as a between-subjects factor, and either ‘orthogonal’ and ‘g band’ (linear accelerations), or ‘rotational plane’ and ‘angular band’ (angular accelerations), as two within-subject factors. Whereas two-way ANOVA is usually undertaken post hoc, after a significant three-way interaction (Lee & Lee, 2018), to address the primary research question and characterise interactions between direction and threshold on duration of accelerations, it was decided a priori to perform two-way ANOVAs on acceleration data. Accordingly, this was undertaken separately for each orthogonal (linear accelerations) and motion plane (angular accelerations), to investigate whether there was a threshold-specific effect of group on duration of accelerations according to direction, and for each threshold (and angular band), to examine whether there was a direction-specific effect of group on duration of accelerations according to threshold.

To compare between groups for capillary blood lactate (three timepoints) and perception of effort post-DM-HIIT exercise (three categories), two-way mixed model ANOVAs, with ‘group’ as the between-subject factor, and ‘serial measure’ as the three level within-subject factors, were used.

Minimum, mean and maximum exercise HR were compared between groups using independent T-tests.

Tests were two-tailed with significance set at 0.05; where correction was applied, to adjust *p* value in post hoc analysis, it is indicated in results.

6.3 Results

6.3.1 Participant characteristics

Sedentary females (SF) were 16.6 ±1.5 y older than female dancers (DF) ($p < 0.001$) and 16.6 ±3.8 kg heavier ($p < 0.001$) and had significantly higher BMI (6.38 ±1.5 $kg.m^{-2}$, $p < 0.001$). Heart rate at rest was lower in SF than DF by 10 ±4 bpm ($p = 0.018$) however, in dancers systolic blood pressure (BP) was lower by 9 ±4 mmHg ($p = 0.024$) and diastolic BP by 15 ±4 mmHg than in sedentary females ($p < 0.001$) (Table 13).

Menstrual status, OC use and daily medication

In DF onset of menses was on average 1.1 ±0.5 y later than in SF ($p = 0.049$); in DF one participant reported that she had not yet begun to menstruate. In DF, 26 % of females ($n = 5$) and in SF 7% ($n = 1$) reported irregular or absent periods; 26% ($n = 5$) of participants in DF and 7% ($n = 1$) in SF reported current OC use. In DF, two participants reported taking daily asthma medication (preventative), and one participant daily insulin for Type 1 diabetes; none of the participants in SF reported taking medication for metabolic or respiratory conditions, however 20% ($n = 3$) reported taking antidepressants, and 7% ($n = 1$) oral medication for hyperthyroidism.

Tobacco and caffeine

In DF 75% ($n = 15$) and in SF 67% ($n = 10$) had never smoked; the number of current smokers ($n = 1$) was the same in each group. In SF, 13% ($n = 2$) and in DF 84% ($n = 16$) identified as 'social only' drinkers; 33% of SF ($n = 5$) reported regularly drinking 6 -10 units per week (highest alcohol consumption band). Caffeine consumption responses indicated 93% of SF ($n = 14$) and 58% of DF ($n = 11$) consumed 1- 5 cups of caffeinated beverages daily, and whilst all sedentary women reported consuming caffeine, 42% ($n = 8$) of dancers indicated they did not drink caffeinated beverages.

Ethnicity

In sedentary females 100% ($n = 15$) and in dancers 68% ($n = 13$) identified 'White' as their ethnic status; in dancers 21% ($n = 4$) identified as 'Oriental' and 11% ($n = 2$) as 'Asian'.

6.3.2 Calcaneal stiffness

Comparing absolute QUS values between groups, left BUA tended to be significantly lower in dancers than in sedentary females by an average of 11 ±5 dB.MHz^{-1} (p = 0.05), and left SI tended to be lower in dancers by 11 ±6 units (p = 0.063). Right calcaneal BUA and left and right SOS, and absolute difference between left and right SI, did not differ between participant groups (Table 16).

In sedentary females, BMI was moderately positively correlated with BUA of the left calcaneus (r = 0.628, p = 0.016) but not of the right (r = 0.126, p = 0.681); there was no relationship between QUS parameters and BMI in female dancers.

Normalising QUS parameters by participant body weight, left and right calcaneal BUA, SOS and SI were significantly higher in dancers than in sedentary females (Table 17).

Table 16 Calcaneal Stiffness (Absolute) (Mean ±SD)

Parameter	DF (n = 19)	SF (n = 15)	p
L SI AU	103±16	114±18	0.063
R SI AU	102±16	106±19	0.513
L-R diff.	10±8	9±10	0.852
L BUA dB.MHz.$^{-1}$	112±16	123±14	0.050
R BUA dB.MHz.$^{-1}$	115±12	115±15	0.923
L SOS m.s^{-1}	1602±39	1615±39	0.340
R SOS m.s^{-1}	1588±39	1606±38	0.217

DF: Female Dancers; SF: Sedentary Females; SI: Stiffness Index; L-R diff.: Absolute difference between L and R Stiffness Index; BUA: Broadband Ultrasound Attenuation; SOS: Speed of Sound.

Table 17 Calcaneal Stiffness (Normalised by Body Weight) (Mean ±SD)

Parameter	DF (n = 19)	SF (n = 15)	p
L SI AU	1.97±0.38	1.68±0.35	0.031
R SI AU	1.96±0.50	1.61±0.39	0.037
L BUA dB.MHz.$^{-1}$	2.14±0.37	1.81±0.30	0.008
R BUA dB.MHz.$^{-1}$	2.19±0.36	1.75±0.38	0.002
L SOS m.s^{-1}	30.55±3.87	23.93±4.23	< 0.001
R SOS m.s^{-1}	30.30±3.97	24.35±3.68	< 0.001

DF: Female Dancers; SF: Sedentary Females; SI: Stiffness Index; L-R diff.: Absolute difference between L and R Stiffness Index; BUA: Broadband Ultrasound Attenuation; SOS: Speed of Sound.

6.3.3 Locomotor profile during DM-HIIT

Linear accelerations

The linear acceleration characteristics during exercise were different between participant groups, as shown by the significant three-way interaction between orthogonal, acceleration band and group (p = 0.041, η^2 = 0.109, orthogonal*g band*group). Two-Way Analysis of Variance, used post hoc to compare directional characteristics between groups according to amplitude, showed a difference in linear profile between 1.5 and 2 g (p = 0.039, η^2 = 0.117, orthogonal*group), whereby duration of vertical accelerations tended to be greater in dancers by 2.4 ±1.2 s (p = 0.052). Above 3 g the distribution of accelerations also differed statistically between groups according to direction (p = 0.026, η^2 = 0.116, orthogonal*group), however, comparison between groups post hoc, within orthogonal, was not significant.

Separate Two-Way Analysis of Variance within each orthogonal did not reveal a directional basis for difference in acceleration profile between participant groups (p > 0.05, g band*group, within ‘forwards’, within ‘sideways’ and within ‘up’) (Figure 14).

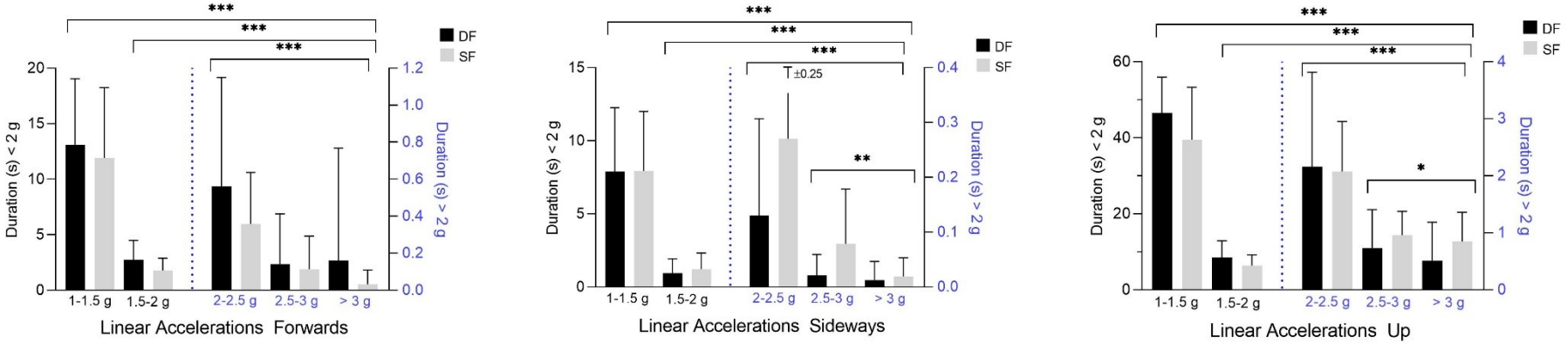
Overall, DM-HIIT performance in both groups produced a diverse linear acceleration profile, as demonstrated by effect size for the interaction between orthogonal and amplitude (p < 0.001, η^2 = 0.883) with longer time spent in vertical than medio-lateral and coronal accelerations during exercise (p < 0.001, η^2 = 0.883, main effect of orthogonal) (Figure 15). Considered as a percentage of total duration of activities prescribed, in sedentary females 49.7 ±17.7 s of dynamic DM-HIIT (10.3 ±3.6% of exercise duration) and in dancers 59.6 ±14.8 s. (12.3 ±3.0%) was spent in vertical acceleration above 1 g (p > 0.05, main effect of group, within ‘up’).

Duration of accelerations was greatest in both groups at the lowest acceleration band (p < 0.001, η^2 = 0.939, main effect of g band): in sedentary females durations by orthogonal were 39.5 ±13.4 s ‘up’, 11.9 ±6.3 s ‘forwards ‘and 7.9 ± 4.1 s ‘sideways’; in dancers: 47.7 ±8.8 s ‘up’, 13.3 ±6.0 s ‘forwards’ and 7.3 ±3.9s ‘sideways’ (p < 0.001, for all pairwise comparisons for duration of accelerations between orthogonals).

A pattern of significantly fewer accelerations performed at each amplitude of acceleration, compared with the amplitude below, was observed

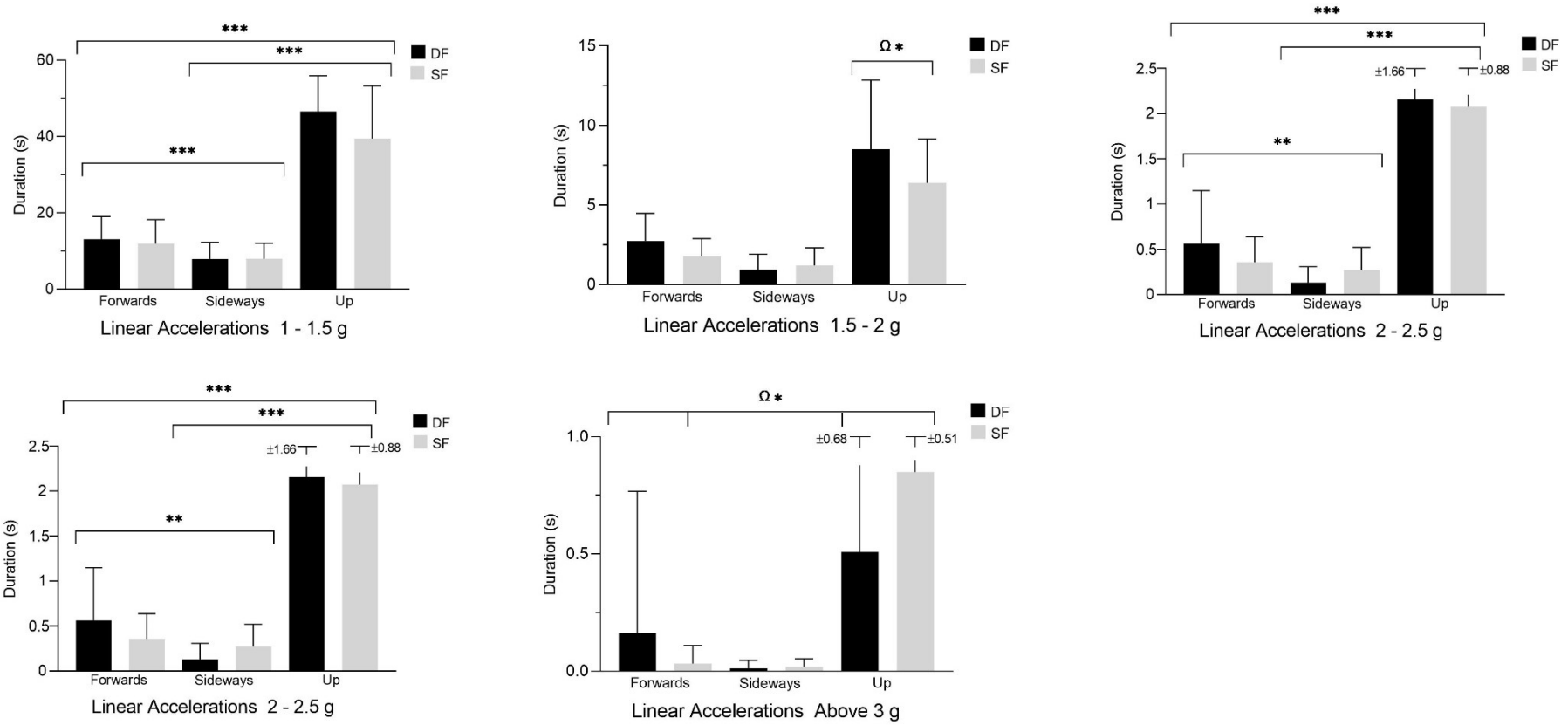
up to a threshold of 2 g ($p < 0.001$, pairwise comparisons between g bands below 2 g), above which no difference was seen ($p = 0.103$, accelerations between 2.5 and 3 g versus above 3 g).

As a percentage of total DM-HIIT duration (485 s dynamic and 305 s static actions, and an additional 70 s standing transitions), sedentary females spent 12.3 ±3.0% and dancers 10.3 ±3.6 % ($p > 0.05$) in accelerations above 1 g. The protocol was otherwise characterised by accelerations below 1 g and steady state (i.e. no acceleration) activity.



DF: Female Dancers; SF: Sedentary Females.
***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$; difference between duration of accelerations by g band.

Figure 14 Linear Accelerations During DM-HIIT Characterised by Orthogonal (Mean ±SD)



DF: Female Dancers; SF: Sedentary Females.
*** $p < 0.001$ ** $p < 0.01$; significant effect of orthogonal on duration; Ω * Significant group*orthogonal interaction effect ($p < 0.05$).

Figure 15 Linear Accelerations During DM-HIIT Characterised by Threshold (Mean ±SD)

Angular accelerations

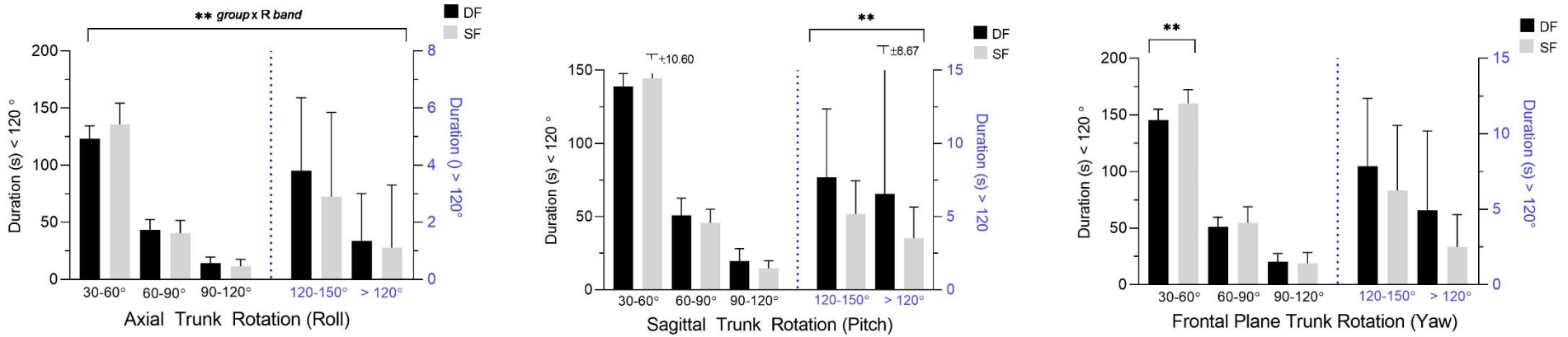
Whilst the three-way interaction between group, rotation plane and angular acceleration band was not significant ($p = 0.165$, $\eta^2 = 0.052$), dancers and sedentary females did exhibit differences in rotational characteristics according to acceleration band ($p < 0.001$, $\eta^2 = 0.319$, group*angular acceleration band), and main effects were observed for both angular plane ($p < 0.001$, $\eta^2 = 0.557$) and for acceleration band ($p < 0.001$, $\eta^2 = 0.995$). Therefore, in order to characterise angular acceleration profile more completely, and to provide consistency with analyses for linear acceleration data, gyroscope data were analysed by rotational plane and by acceleration band, using separate Two-Way Repeated Measures ANOVAs.

The biggest difference between the two groups (according to effect size) was observed in the frontal plane ($p < 0.001$, $\eta^2 = 0.287$, group*Rband, within 'yaw') (Figure 16): sedentary females performed 14.6 ±3.9 s more side-bending trunk accelerations during DM-HIIT in the lowest Rband (30 to 60 rad.s^{-2}) compared with dancers ($p = 0.004$ pairwise comparison). Sagittal plane accelerations (i.e. trunk flexion-extension) also differed between the groups ($p = 0.005$, $\eta^2 = 0.151$, group* Rband, within 'pitch'). Whereas in dancers the difference in duration between the two highest Rbands (1.1 ±1.1 s) was not significant ($p > 0.999$), sedentary females spent 1.6 ±0.3 s less in accelerations above 150 rad.s^{-2} compared with the Rband immediately below (120 to 150 rad.s^{-2}) ($p < 0.001$). In the axial plane, whilst the interaction term was significant ($p = 0.004$, $\eta^2 = 0.188$, group* Rband, within 'roll'), post hoc comparison did not reveal a statistically significant difference between participant groups at any level of Rband (Figure 16).

In each plane of trunk motion, pairwise comparison revealed a statistically significant reduction in duration of accelerations within each angular acceleration band compared with the one immediately below ($p <0.001$), and durations were longer in the lowest two angular acceleration bands (i.e. < 90 rad.s^{-2}) in all three planes. In sedentary females durations were 176.0 ±27.1 s for left-right rotation, 190.2 ±17.2 s for flexion-extension, and 214.8 ±23.5 s for side-bending; in dancers corresponding durations were: 166.7 ±19.5 s, 189.8 ±19.2 s and 196.7 ±16.6 s, respectively.

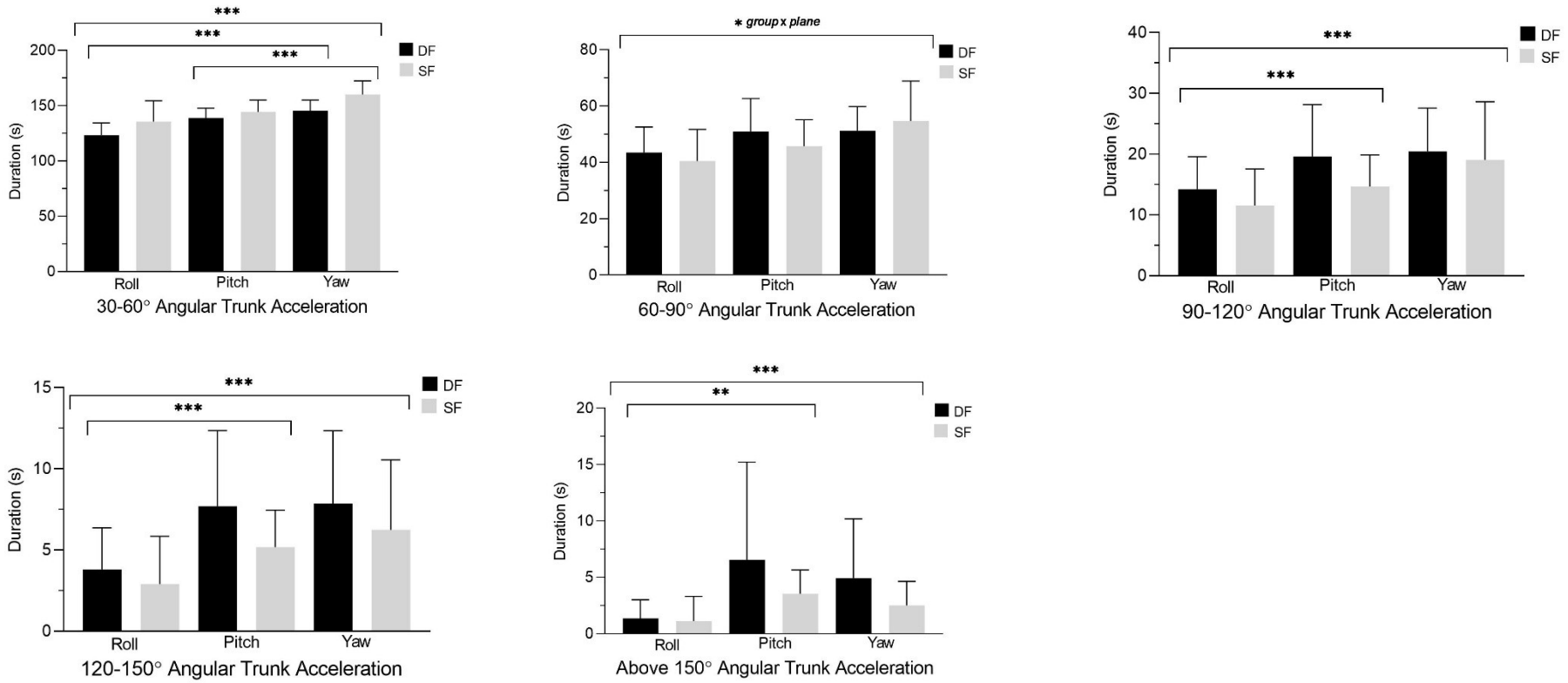
Angular accelerations above 30 rad.s^{-2} comprised 44.4 ±5.9% of the protocol in sedentary females and 44.5 ±4.6% in dancers ($p > 0.05$), with the

remainder of protocol duration spent in angular trunk accelerations below this threshold, or in constant velocity angular trunk motion.



DF: Female Dancers; SF: Sedentary Females; R band: Radian Band
** $p < 0.01$.

Figure 16 Duration of Angular Accelerations During DM-HIIT By Plane (Mean ±SD)



DF: Female Dancers; SF: Sedentary Females.
*** $p < 0.001$ ** $p < 0.01$; significant effect of orthogonal on duration; Ω * Significant group*orthogonal interaction effect ($p < 0.05$).

Figure 17 Duration of Angular Accelerations (Mean ±SD)

6.3.4 Physiological responses to DM-HIIT

Exercise heart rate

Peak heart rate (HR_{PEAK}) during DM-HIIT was 10 ±4 bpm higher in dancers than sedentary females (p = 0.017) (Table 18). As a percentage of HR_{PEAK} recorded during DM-HIIT, mean HR during exercise was >80% HR_{PEAK} for 75 ±18% of the protocol in DF and 72 ±21% in SF (p = 0.665) and > 90% HR_{PEAK} for 47 ±20% of exercise in DF, and 40 ±18% in SF, respectively (p = 0.353).

Table 18 Heart Rate During DM-HIIT (Mean ±SD)

Characteristic	DF (n = 17)	SF (n = 14)	p
HR_{MIN} bpm	106 ±20	98 ±18	0.308
HR_{MEAN} bpm	168 ±17	158 ±11	0.080
HR_{MAX} bpm	194 ±12	184 ±9	0.017
>80% $HR_{PEAK-REC}$ s	644 ±157	618 ±176	0.665

$HR_{PEAK-REC}$ denotes peak heart rate recorded during exercise.

Capillary blood lactate

Capillary blood lactate concentration (BLa) in both groups was significantly elevated immediately after the protocol, compared with after submaximal exercise (p < 0.001, η^2 = 0.507, main effect of time) (Table 19), increasing by 4.0 ±0.6 mmol.L^{-1} (p < 0.001) and remaining 2.6 ±0.5 mmol.L^{-1} above BLa after submaximal exercise two minutes after DM-HIIT was completed (p < 0.001).

Whilst there was no significant main effect of group for lactate response to DM-HIIT, in seven dancers BLa values immediately after DM-HIIT were ≥ 10 mmol.L^{-1}, accounting for wider dispersal of BLa results in DF.

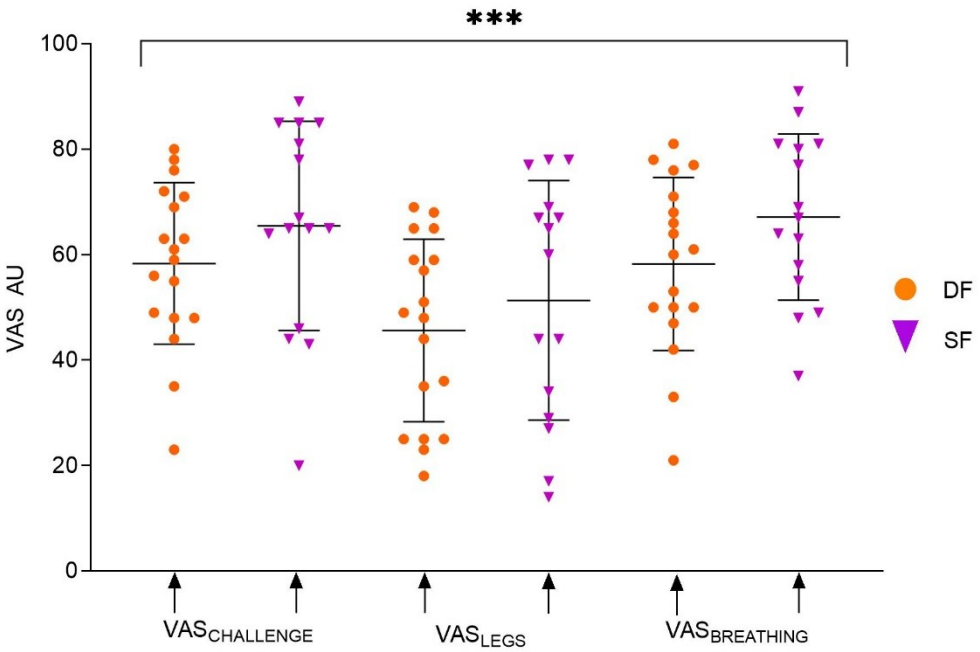
Table 19 Lactate Concentration After Submaximal Exercise and DM-HIIT (Mean ±SD)

	DF (*n* = 17)			SF (*n* = 14)			Time*group	
Capillary BLa	S-Max	DM-HIIT	+2 min	S-Max	DM-HIIT	+2 min	***p***	η^2
mmol.L^{-1}	3.8 ±1.9	7.6 ±4.1	5.4 ±3.5	4.1 ±1.6	8.3 ±1.0	7.6 ±1.2	0.146	0.061

DF: Female Dancers; SF: Sedentary Females; BLa: Capillary blood lactate.

Perception of effort

Perception of demand imposed by DM-HIIT did not differ between intervention groups (p = 0.902, η^2 = 0.003; group*VAS$_{CATEGORY}$). However, exercise challenge and cardiorespiratory demand were rated higher than demand on legs (p < 0.001, η^2 = 0.250; main effect of VAS$_{CATEGORY}$) (Figure 18); according to pairwise comparison, overall challenge of DM-HIIT was rated 13 ±4% higher (p = 0.004), and breathing demand 14 ±4% higher than the demand on legs (p = 0.003).



DF: Female Dancers; SF: Sedentary Females.
*** Main effect of VAS$_{CATEGORY}$ (p < 0.001)

Figure 18 Perception of Exercise Challenge

Lacate, perceived effort and accelerations

The strongest relationship between $VAS_{CATEGORY}$ and a physiological measure of exercise demand was observed between VAS_{LEGS} and capillary blood lactate (BLa) concentration immediately after DM-HIIT, which were moderately but not significantly positively correlated ($p = 0.066$; $r = 0.324$). No relationship between BLa post-DM-HIIT and $VAS_{CHALLENGE}$ ($p = 0.541$, $r = 0.110$) or $VAS_{BREATHING}$ ($p = 0.202$, $r = 0.201$) was found.

Accelerations above 3 g (sum of durations in all three orthogonals) and BLa immediately after DM-HIIT were significantly positively correlated ($p = 0.036$, $r = 0.360$), and a positive relationship was also observed for total accelerations above 2 g, however this was not statistically significant ($p = 0.263$, $r = 0.198$). The highest BLa recorded after DM-HIIT (14.3 $mmol.L^{-1}$) was observed in a female dancer, for whom total duration of accelerations above 3 g (5.6 s) and 2 g (15.9 s) were highest out of all participants.

6.3.5 Locomotor profile and physiological responses to submaximal exercise

Linear acceleration profile

Linear acceleration profile differed between sedentary females and dancers during submaximal exercise ($p < 0.001$, $\eta^2 = 0.329$, orthogonal*g band*group), and effect sizes for orthogonal ($p < 0.001$, $\eta^2 = 0.907$) and g band ($p < 0.001$, $\eta^2 = 0.931$) were large. Analysing accelerations within each orthogonal, according to the approach used for DM-HIIT, vertical acceleration characteristics were different between intervention groups ($p < 0.001$, $\eta^2 = 0.348$, group*g band, within ‘up’); duration of vertical accelerations above 2 g was 2.7 ±0.6 s longer in dancers ($p < 0.001$, pairwise comparison between groups) but forwards and sideways accelerations were not different ($p > 0.05$, group*g band, within ‘forwards; ‘sideways’).

Angular acceleration profile

Trunk angular acceleration profile during submaximal exercise also differed between dancers and sedentary females ($p < 0.001$, $\eta^2 = 0.203$, group*plane*Rband) and there were large main effects for angular plane ($p < 0.001$, $\eta^2 = 0.734$) and for Rband ($p < 0.001$, $\eta^2 = 0.975$). Adopting the approach used for linear accelerations, a difference between groups was found

in the frontal plane ('yaw') ($p < 0.001$, $\eta^2 = 0.304$; group*Rband within 'yaw'), duration of accelerations during trunk side-bending was longer in DF than SF (22.7 ±3.9 s versus 15.5 ±5.1 s, $p < 0.001$). Duration of angular rotations above 90 rad.s^{-2} was also greater in DF than SF ($p = 0.001$, $\eta^2 = 0.203$, group*plane, above 90 rad.s^{-2}) by.0 ±0.8 s ($p < 0.001$).

Heart rate during submaximal exercise

No differences between dancers and sedentary females were observed in mean, minimum and peak HR during submaximal exercise (Table 20). Data from four participants (three dancers and one sedentary female) were not available for analysis, due to loss of signal (intermittent drop out).

Table 20 Submaximal Exercise Heart Rate (Mean ±SD)

Characteristic	DF (*n* = 16)	SF (*n* = 14)	*p*
HR_{MIN} bpm	82 ±4	86 ±4	0.838
HR_{MAX} bpm	169 ±2	165 ±4	0.353
HR_{PEAK} bpm	140 ±2	137 ±3	0.466

DF: Female Dancers; SF: Sedentary Females.

Lactate concentration

Capillary BLa concentration immediately after submaximal exercise was 3.8 ±1.9 mmol.L^{-1} in dancers and 4.1 ±1.6 mmol.L^{-1} in sedentary females, the difference between groups was not statistically significant.

6.4 Discussion

Evidence from the present investigation, which supports results obtained previously in sedentary females (Chapter 5), demonstrated that diverse HIIT, constrained to 3 x 3 m exercise area, provided an acute stimulus to vertical acceleration in both sedentary females and ballet dancers.

In sedentary females (SF), duration of accelerations above 1 g in the vertical direction was 49.7 ±17.7 s and in female dancers (DF) 59.6 ±14.8 s, corresponding to 10.3 ±3.6% and 12.3 ±3.0% of total duration for dynamic actions during the protocol (485 s). Compared with percentages for durations above 1 g forwards (2.9 ±1.5% and 3.5±1.8%) and sideways (2.0 ±1.1% and 1.7 ±1.0%) in SF and DF, results indicated that the locomotor stimulus from exercise actions was predominantly vertical in axis of orientation, as supported by the statistical difference in duration of accelerations between orthogonals.

Accompanying these findings, analysis of heart rate during DM-HIIT provided evidence that participants exercised at high intensity, characterised as percentage of total protocol duration (860 s) spent ≥ 90% of peak exercise heart rate. In SF, this amounted to 40 ±18% % of DM-HIIT and in DF 47 ±20% and was not significantly different between intervention groups ($p > 0.05$). Evidence from heart rate that DM-HIIT elicited high-intensity effort was further supported by a large and highly significant elevation in capillary blood lactate (BLa) concentration of 4.0 ±0.6 mmol.L^{-1} immediately after DM-HIIT, compared with BLa after a preceding bout of submaximal diverse shuttle runs, and indicated diverse actions prescribed in the protocol imposed high metabolic demands on muscle tissue in both exercise groups.

As well as quantifying locomotor profile and describing acute physiological demands of a single training bout, a principal aim of this investigation was to examine the osteogenic potential of the protocol, which featured multidirectional activities ecologically evidenced in team sport environments (Taylor et al., 2017). Ground reaction forces engendered by diverse exercise actions, such as brief high-speed running, jumping and kicking, which were included in the protocol, are proposed to elicit osteogenic effects on the femur and pelvis (Guadalupe-Grau, Fuentes, Guerra, & Calbet, 2009). This has been demonstrated in an applied context in recreational team sports interventions, which have demonstrated site-specific increases in BMD at the

femur and hip in females exposed to in recreational football (Krustrup, Helge, et al., 2018), and increases in whole-body BMD in older males and females (Skoradal et al., 2018).

Therefore, evidence that locomotor profile during DM-HIIT was predominantly characterised by accelerations in the vertical axis, speculatively attributable to frequent landing impacts from exercise actions prescribed in the protocol, supports a hypothesis of potential pro-bone stimulus for the protocol. For example, during DM-HIIT participants performed 10 s all-out vertical jumping and four phases (105 s in total) of multidirectional hopping, which as well as vertical travel required lateral and sagittal progression, effected by a propulsive exchange between landing and reacceleration leg at the edge of the grid. In a recent study in postmenopausal women, which ranked the osteogenic potential of a range of activities, highest compressive and tensile strains on the femur were experienced during the propulsion phase of hopping (Pellikaan, Giarmatzis, Vander Sloten, Verschueren, & Jonkers, 2018), which indicates that actions included in DM-HIIT had the potential for eliciting high mechanical strains. This is further supported by a 6 month intervention that administered brief episodes of high-intensity actions, which reported increased femoral neck BMD in collegiate females after 10 maximal jumps 3 x weekly (Kato et al., 2006). In the context of these findings, it may be speculated that a localised osteogenic effect at the hip and femur could occur after longer-term DM-HIIT practice. However, according to the mechanostat proposal for bone adaptation (Frost, 1987), high-magnitude and versatile mechanical strains, arising from impacts and diverse actions scheduled during exercise, would be required to exceed prevailing conditions of background loading and habitual PA in premenopausal females.

In the present study, we did not quantify ground reaction force directly, and therefore impacts can only be inferred from locomotor data, particularly as the motion sensor unit did not distinguish between vertical accelerations accompanying a flight phase and descent, from those generated during vertical trunk displacements not associated with impact. Despite these limitations to the present study, it has been demonstrated in adults and older populations that acceleration profile is adequate to provide a surrogate for measuring stimulus intensity from daily physical activity (PA) (Chastin et al., 2014), and to assess

long-term dose-response outcomes in regional BMD in premenopausal females (Jämsä et al., 2006). From population-based evidence derived from habitual PA, a threshold of 3.9 g has been proposed for osteogenic effects of daily acceleration exposure at the hip and pelvis (Vainionpää et al., 2006), and a threshold of 3.9 g for improvement in femoral bone geometry (Ahola et al., 2010). For the purpose of comparison between the latter studies and the present one, duration of high-magnitude (> 3 g) accelerations during DM-HIIT were multiplied by unit sampling rate (100 Hz) to provide counts of high-intensity accelerations. Accordingly, durations of 0.8 ±0.5 s in SF and 0.5 ±0.7 s in DF, corresponding to 85 ±51 episodes of vertical accelerations > 3 g in SF and 50 ±68 in DF, were observed during DM-HIIT. In the context of what has been reported previously in premenopausal women, locomotor profile results suggest DM-HIIT provided a focussed dose of high-magnitude accelerations. However, in volume, this did not exceed 100 peak accelerations (the dose positively correlated with greater BMD at the femoral neck, trochanter and Ward's triangle), proposed to be osteogenic from wrist-worn accelometric examination of daily PA (Jämsä et al., 2006; Vainionpää et al., 2006). In the latter studies, threshold for high-magnitude accelerations was > 3.9 g, which is higher than the upper threshold categorised during DM-HIIT. It may therefore be speculated that whilst counts, as opposed to durations, of high-threshold (i.e. > 3 g) accelerations during acute DM-HIIT approached values proposed for site-specific osteogenic effects, intensity (i.e. magnitude of peak accelerations) may be insufficient to elicit adaptation in bone with chronic training. Lack of discrete evidence for accelerations ≥3.9 g exhibited here during DM-HIIT suggests the protocol should be modified in future implementation. As magnitude of strain has been shown to be a critical determinant of bone responses (Rubin & Lanyon, 1985; Turner, 1998), further phases of jumping at maximal intensity could be included, as well as additional bouts of lateral cutting and diagonal progressions within the grid, given evidence in premenopausal women that mechanical strains delivered via high- and odd- (i.e. diverse) impacts provide an anabolic stimulus to regional bone adaptation at the hip and femur (Martyn-St James & Carroll, 2010).

However, in support of the HIIT approach adopted in the present protocol, a cross-sectional study, which investigated dose-response effects for daily accelerations on bone, found that even brief exposure to HI activities was

positively correlated with BMD T score in pre- and postmenopausal women (Rowlands et al., 2019). Analysis of the population data accessed by Rowlands and colleagues showed that in premenopausal females, spending 1 – 2 min, or ≥2 min, within daily PA at intensities surpassing ≥ 1000 mg, as assessed by wrist-worn accelererometry, was positively associated with BMDT score, and with calcaneal SOS, an indicator of bone elasticity, and BUA, an indicator of bone stiffness (Stiles, Metcalf, Knapp, & Rowlands, 2017). According to PA categories described in milligraviational (mg) equivalents by Stiles et al., duration of maximal intensity DM-HIIT, which comprised 145 s of 485 s dynamic exercise actions, met the upper threshold of volume prescription (≥2 min) for an osteogenic effect of brief, high-intensity activity (1000 mg [0.001 g]) in premenopausal females (Stiles et al., 2017). This offers further support for the proposal that whilst volume of high-intensity exercise included in the protocol was potentially adequate, performance did not elicit intensities of acceleration signal, demonstrated to be osteogenic in this population, under the conditions examined.

Comparison between the present study and those of Rowlands et al., and Stiles et al., is not straightforward. The motion sensor unit was positioned on the posterior trunk during DM-HIIT, and therefore quantified vertical trunk motion against, and assisted by, gravity, whereas wrist-worn accelerometers, as used in the study by Stiles et al., characterised appendicular upper limb accelerations. Furthermore, condition of PA, which for DM-HIIT was externally prescribed in untrained participants, is not directly comparable with habitual PA under conditions of free-living. Furthermore, there are fundamental differences in approach to treatment of acceleration data, which was characterised for DM-HIIT by orthogonal and duration, whereas in these other studies, data were averaged, across 1 s epochs, to provide a scalar value derived from triaxial accelerations.

An advantage of the present approach to characterising locomotor profile is that it enabled direction, as well as magnitude, of accelerations to be characterised during acute exercise. In contrast, previous approaches to accelerometric assessment of PA have integrated orthogonal characteristics within a scalar value (Karantonis et al., 2006; Yang & Hsu, 2010), which reduces dimensionality and therefore directional specificity. Other researchers

have examined peak vertical accelerations and described osteogenic thresholds for activity (Heikkinen, Vihriala, Vainionpaa, Korpelainen, & Jamsa, 2007; Jämsä et al., 2006; Vainionpää et al., 2006). It was considered important here to retain representation of orthogonal characteristics during analysis, according to the hypothesis that vertical accelerations associated with impacts, and lateral accelerations from lateral cutting, could deliver compressive and tensile strains during MI and HI phases of DM-HIIT. It is this pattern of exercise-induced mechanical strain that has been proposed to elicit bone anabolic responses, as a result of mechanobiological signalling arising from axial compressions and whole bone deformations (Hart et al., 2017; Tyrovola & Odont, 2015).

In the present study, BLa concentration demonstrated the protocol was a significant stimulus to anaerobic muscle metabolism. Aside from quantifying exercise intensity in terms of metabolic demand, this finding could also be interpreted as indicating other potentially osteogenic effects of diverse HIIT, which were uncharacterised but are indirectly supported by the elevation in blood lactate. Mechanical effects of muscle contractions on bone are well-described (Hart et al., 2017), and evidence of myokine-mediated muscle-bone interaction suggests the influence of muscle actions on bone exceed the mechanical (Bonewald, 2019; Cianferotti & Brandi, 2014). During diverse HIIT, along with vertical jumping and flighted progressive actions, whole-body deceleration and reacceleration were frequent during COD at the edge of the grid, necessitating eccentric lower limb contractions. It has been shown in vivo that bi-articular muscles, which span the pelvis, femur and shank, generate high tensile forces to counteract bending moments generated during impacts and singl-leg stance (Lu et al., 1997), both of which were featured during activities in the protocol. It may therefore be speculated that the elevation in blood lactate could also have resulted from muscle contractions counteracting impact-associated compressive strains and bending moments from frequent COD.

According to peripheral indicators of metabolic demand and exercise heart rate (HR), results suggested that DM-HIIT elicited similar responses to those seen in team sports, intermittent high-intensity training and dance performance. Immediately after DM-HIIT, BLa was 8.3 ±1.0 $mmol.L^{-1}$ in SF and 7.6 ±4.1 $mmol.L^{-1}$ in DF, and was significantly elevated by 4.0 ±0.6 $mmol.L^{-1}$ compared with BLa after the brief bout of submaximal exercise that preceeded

DM-HIIT. These values in SF and DF are equivalent to results reported in laboratory assessment of BLa after 75% of 21 min maximal intensity sprint interval training (SIT) and 75% of 23 min high volume HIIT ($HIIT_{HV}$) in young men and women (Olney et al., 2018).

Furthermore, mean exercise HR (HR_{MEAN}) during DM-HIIT, which was 158 ±11 bpm in SF, and tended to be higher (168 ±17 bpm) in DF, was similar to exercise HR after 75% of $HIIT_{HV}$, and slightly above the value graphed for 75% of SIT (Olney et al., 2018). In comparison with BLa and HR responses to the team sports actions in the present protocol, peak BLa (5.2 ±0.6 $mmol.L^{-1}$) and HR_{MEAN} (161 ±2 bpm) were lower after 60 minutes 7 vs 7 football in untrained males (Randers et al., 2010). Similarly, in a recent crossover study in young (20 y) females, peak BLa (2.5 ±1.1 $mmol.L^{-1}$) and HR_{MEAN} (144 ±11 bpm) reported for 60 minutes netball were lower than in DF and SF for DM-HIIT (McIver, Greig, & Marrin, 2019). Results from crossover trials with the other sports codes examined showed that BLa and HR_{MEAN} in DF and SF were also higher than BLa (2.2 ±1.4 $mmol.L^{-1}$) and HR_{MEAN} (130 ±14 bpm) reported for 60 minutes aerobics, and BLa (5.6 ±2.6 $mmol.L^{-1}$) and HR_{MEAN} (150 ±9 bpm) for 60 minutes indoor interval cycling (McIver et al., 2019). Furthermore, comparing acute physiological responses to DM-HIIT, which included lateral progressions and frontal plane footwork from court-based team sport, BLa in SF and DF, and HR_{MEAN} in DF, were more elevated during DM-HIIT than HR_{MEAN} (165 ±9 bpm) and Bla (5.2 ±2.7 $mmol.L^{-1}$) in collegiate females for a bout of competitive basketball (Matthew & Delextrat, 2009). Finally, compared with results in elite sport dancers, BLa in SF and DF after DM-HIIT was higher than after a preliminary competitive dance bout (6.9 ±2.6 $mmol.L^{-1}$), and higher in SF, but lower in DF, than after a subsequent dance bout (8.0 ±2.1 $mmol.L^{-1}$) in females competing at high level (Bria et al., 2011). In contrast, BLa in female ballet dancers after a 70 minute ballet class (11 $mmol.L^{-1}$) (Guidetti, Gallotta, Emerenziani, & Baldari, 2007) was higher than in either SF or DF after DM-HIIT.

These results suggest that DM-HIIT, applied acutely in the two groups of females examined, successfully replicated environmental demands in exercising muscle evidenced in high-intensity intermittent exercise. A single bout of 14.5 min DM-HIIT was more taxing than 60 min of either SSG in sedentary males, netball in recreationally active females, and basketball

matchplay in collegiate females, and resulted in similar BLa post-exercise to results in competitive dance at elite level, but was lower than BLa reported in young females after 70 minutes classical ballet training. Since team sports have been shown to generate an osteogenic stimulus in different female populations (Krustrup, Helge, et al., 2018), and in light of comparable physiological demands, one could extrapolate to suggest osteogenic effects for DM-HIIT. However, such extrapolation should be undertaken with caution. There are a number of limitations to capillary blood lactate as a proxy of metabolic demand of exercise on muscle, not least that blood lactate concentration represents the balance between muscle lactate production and efflux to blood and lactate uptake into non-exercising muscle and other tissues. In addition, work to rest ratio, prescribed in DM-HIIT as 1 : 0.6 for duration (s) of dynamic : balance/mobility actions, affects accurate estimation of overall metabolic burden, which is a limiting factor for workload comparison between non-standardised exercise formats (Torma et al., 2019).

The rationale for prescribing activities within a limited exercise area during DM-HIIT was to increase the density of exercise actions eliciting high effort, associated with whole-body deceleration and reacceleration. Participants were instructed to use the grid edges to reverse movement direction, or change plane of motion during whole-body actions, to increase COD frequency and provide a focussed, adaptogenic stimulus. Constraining performance area in this manner has the disadvantage of reducing velocities that can be achieved during progressive actions, such as sagittal runs and lateral cutting, and could account for low durations of accelerations at high-intensity (> 3 g). Nevertheless, it may be speculated that the limited floorspace elevated exercise workload, in the context of what has been demonstrated for high-intensity actions (HIA) in teamsports (Bourdon et al., 2017).

For example, a typical deceleration-acceleration epoch of 1.5 s for HIA has been identified in a time-motion analysis in netball (Sweeting et al., 2017), and in elite female footballers, time-motion analysis of activity profile during SSG found an average ratio of 4 s for duration of HIA, followed by a period of 48 s low intensity activity (LIA), i.e. a 1:12 ratio for HIA:LIA overall (Gabbett & Mulvey, 2008). Compared with these findings, the ratio of HIA:LIA of 1:4 s applied during DM-HIIT, which comprised eight phases of HIA, followed by

intervals of LIA and static balancing tasks, suggests that HIA was more densely prescribed than during SSG, and supports the hypothesis that increased exercise intensity caused the significant elevation in capillary blood lactate concentration observed in both participant groups.

It should be acknowledged, however, that evidence from time-motion analysis in team sports is substantially representative of performance in elite populations, who are long-term familiarised with activities within their performance code, and therefore not directly comparable with locomotor responses in the interventional groups who undertook a one-off bout of training in the present study. Although participants were offered audiovisual familiarisation (see Methods 6.2.8), they were exposed naively to DM-HIIT, and in the basal state were either sedentary, or long-term ballet trained. It may therefore be hypothesised that factors such as pre-existing exercise experience, and lack of long-term familiarity with HI exercise actions, could also have affected the potential of DM-HIIT to elicit high-magnitude accelerations. This could explain the highly significant main effect for amplitude of accelerations, which showed that in both SF and DF during DM-HIIT duration of accelerations was greatest at the lowest magnitude (1 -1.5 g) characterised. As an interpretion of results for a one-off bout of training, speculation of a potential dose-response effect of chronic exercise on high-magnitude accelerations during DM-HIIT could be further examined in a longer-term training scenario. In accordance with the previously cited evidence of threshold-specific osteogenic effects in premenopausal females (Stiles et al., 2017), quantifying high-magnitude accelerations (> 3 g), before and after longer-term training, could yield insight into whether HIIT applied in this format provides a stimulus to locomotor adaptation, and enhances capacity to exercise at intensities associated with anabolic effects on bone.

6.4.1 Limitiations

A limitation of the present study is that acceleration profile could have been affected by the differences in compliance between the surfaces on which training was conducted. Dancers performed DM-HIIT on a sprung floor, which is likely to have absorbed some of the force generated during foot contact and attenuated landing impact, whereas sedentary females trained on a hard

surface in an exercise studio. This could be addressed in future studies by standardising exercise environment during bouts of training.

A further potential source of limitation is that capacity to perform exercise, in response to an audiovisual presentation, could have affected locomotor output and training intensity. It is possible that inter-individual differences in preferred learning style and motor skill attenuated exercise intensity and affecting physiological responses; for example, if participants were unsure of choreography, and therefore did not complete tasks as prescribed. This effect could be mitigated by providing additional familiarisation prior to exercise testing, and by using a standardised protocol as a test-retest method for characterising responses to longer-term DM-HIIT.

6.4.2 Conclusion

Results for brief diverse HIIT are encouraging. Despite fundamental differences in context for high-intensity actions between DM-HIIT and team sports codes, from which exercise actions were derived, capillary blood lactate concentration and heart rate responses to DM-HIIT suggested that constraining exercise area was effective in elevating physiological demands of exercise.

From the perspective of bone anabolic potential for the protocol, which was the principal aim under investigation, participants' locomotor profile provided evidence of predominantly vertical accelerations during exercise, which were attributed to frequent take off and landing associated with exercise actions, but could also have been caused by axial trunk displacements during non-impact activities. A key finding was that duration of higher-magnitude acclerations, hypothesised to constitute the signal that promotes bone adaptation, was inadequate in the context of what has been demonstrated for an osteogenic effect of PA in premenopausal females.

Therefore, longer-term training studies are required to examine whether chronic administration of DM-HIIT, modified to increase duration of high-intensity actions involving impacts, could elicit a training effect on locomotor characteristics. This could elucidate whether brief exercise in the format of DM-HIIT has the potential to promote bone adaptation, by enhancing capacity to perform vertical accelerations, during diverse actions and multidirectional whole-body COD performed at high frequency.

7 Effects of 12 Weeks Diverse HIIT On Bone: A Randomised Controlled Home Exercise Intervention in Sedentary Premenopausal Females

7.1 Introduction

Inactivity has been proposed to contribute to 6 – 10% of major, non-communicable diseases (Lee et al., 2012), and yet sedentary behaviour (SB), which denotes activity in the range of 1 – 1.5 METs (Metabolic Equivalents) (Ainsworth et al., 2000), is increasingly common in the UK (Heron, Neill, McAneney, Kee, & Tully, 2019). Inactivity may directly contribute to poor health outcomes by influencing metabolic precursors of the so-called lifestyle-induced chronic diseases (LICD) (Owen, Healy, Matthews, & Dunstan, 2010). In cross-sectional examination, strong, positive associations have been found between SB and type 2 diabetes mellitus (T2DM) and metabolic syndrome (Van der Berg et al., 2016), a cluster of conditions which includes dyslipidemia, hypertension and insulin resistance (Eckel, Grundy, & Zimmet, 2005). Moreover, SB has been strongly linked to increased waist circumference (an indicator of visceral adiposity) and increased fat mass (Myers, Gibbons, Finlayson, & Blundell, 2017), and thus contributes to systemic inflammation (Dandona, Aljada, & Bandyopadhyay, 2004; Monteiro & Azevedo, 2010).

Local and systemic markers of inflammation, such as interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and C-reactive protein (CRP), which are elevated in association with obesity (Guri & Bassaganya-Riera, 2011), may exert deleterious effects on bone, by promoting osteoclast activity and inclining bone turnover towards bone resorption (Cao, 2011). For example, osteocalcin, a biomarker of bone formation, was found to be inversely associated with high-sensitivity CRP in overweight and obese premenopausal females (Lucey et al., 2013), and in young obese females, markers of bone formation were significantly decreased compared with controls. Interestingly, in this study formation markers were reduced similarly in anorexic (AN) and obese participants (Maïmoun et al., 2020). Evidence that pro-inflammatory markers, notably TNF-α and Interleukin(IL)-6, are elevated in AN similarly to in obesity (Solmi et al., 2015) could provide a causal link to explain similarly reduced formation in both conditions, despite contrasting states of adiposity.

Evidence that the ‘energy excess’ condition of obesity affects bone metabolism (Pérez et al., 2016), and that in the ‘energy deficient’ condition of anorexia bone resorption and formation are uncoupled (Idolazzi et al., 2018), supports a proposal of significant cross-regulation between bone and energy metabolism (Confavreux, Levine, & Karsenty, 2009; Fernandes, Gonçalves, & Brito, 2017). Studies investigating the bone formation marker osteocalcin (OC), speculated to also influence glucose metabolism via hormone-like action (Booth, Centi, Smith, & Gundberg, 2013; Kanazawa, 2015), have found OC is inversely related to fat mass and plasma glucose in older males and females (Kindblom et al., 2009; Lee, Jo, Kim, Kim, & You, 2015) and central adiposity in postmenopausal women (Movahed et al., 2012). Accordingly, interventions which address the obese phenotype and aim to reduce truncal adiposity may also influence bone, by addressing dysregulation of bone formation associated with reduced osteoblastic expression of osteocalcin in the hyperglycemic state (DeLuccia, Cheung, Ramadoss, Aljahdali, & Sukumar, 2019). It therefore follows that physical activity may exert effects on the skeleton not fully accounted for by the paradigm of osteogenic adaptation mediated by mechanosensory responses to load. In the light of this proposal, alongside the stimulus to osteogenesis elicited by skeletal loading, prescribing exercise to decrease adiposity could improve bone outcomes by reducing the deleterious impact of pro-inflammatory cytokine activity on bone formation-resorption coupling (Gleeson et al., 2011; Pagnotti et al., 2019).

Nevertheless, although exercise has also been promoted to mitigate against so-called lifestyle-induced chronic diseases (LICD) (Pedersen & Saltin, 2006) and address parallel increases observed in occupational SB obesity and overweight (Church et al., 2011), it is estimated that 31% of the population worldwide are inactive, defined as engaged in less than 30 min moderate-intensity exercise 5 days per week (Hallal et al., 2012). Moreover, population evidence of a dose-response association between number of perceived barriers to exercise and level of PA (Reichert, Barros, Domingues, & Hallal, 2007) suggests that disinclination towards PA could be mitigated by better formats of exercise delivery and accessibility.

In recent years, as qualitative evidence in different age-groups and populations has shown ‘lack of time’ is a frequently cited barrier to PA (Al-

Hazzaa, 2018; Bautista, Reininger, Gay, Barroso, & McCormick, 2011), high-intensity interval training (HIIT) has been investigated as a possible approach to reduce duration and increase acceptability of exercise (Biddle & Batterham, 2015; Gibala, Gillen, & Percival, 2014). HIIT has been shown to improve indicators of metabolic health (Jelleyman et al., 2015), and elicit significantly greater improvement in VO_{2PEAK} than moderate intensity continuous training (MICT) in patient populations (Weston et al., 2014). However, applying HIIT to target an osteogenic effect is not represented currently in the literature.

Exercise combining impact and resistance components preserves and improves bone in both pre- and post-menopausal females according to meta-analysis (Xu, Lombardi, Jiao, & Banfi, 2016), however disinclination to PA, arising from perceived and/or 'true' time-poverty, could prevent women accessing the benefits to bone that have been demonstrated for exercise participation (Nikander et al., 2010). For example, females who reported 'lack of time' as a perceived barrier to exercise were found to be less likely to meet PA recommendations (Welch, McNaughton, Hunter, Hume, & Crawford, 2009). It may therefore be inferred that reducing exercise duration could be an effective strategy to target sedentary behaviours in women. Therefore, implementing a HIIT approach to deliver impact exercise in sedentary women could provide insight into acceptability and effectiveness of brief exercise to target bone health in this population.

Furthermore, examining the effects of HIIT on inflammatory status and truncal adiposity, alongside indicators of bone metabolism, has the potential to demonstrate how these factors may interact to influence the outcome of a targeted exercise intervention on bone. As previously stated, biomarkers of inflammation have been positively associated with truncal adiposity (Myers et al., 2017), yet whilst positive, exercise-associated effects on systemic inflammation, mediated by reduction in central adiposity, have been explored, the impact on bone is less extensively described. The relationship between truncal fat mass, inflammatory status and biomarkers of bone metabolism in response to HIIT has not been characterised in sedentary females, yet this could provide insight into whether brief exercise, at sufficiently high intensity, could benefit bone in the premenopause by lowering inflammatory status.

In a previous 12 week study in pre- peri- and post- menopausal women (Chapter 5), we demonstrated high acceptability and compliance with a novel HIIT protocol, based on high-impact and directionally diverse participatory actions demonstrated to occur frequently in team sports (Randers et al., 2010). Whilst the primary aim, of examining feasibility of this approach to provide an osteogenic exercise stimulus, was successfully achieved, participants differed in menopausal status and use of hormone replacement therapy, which confounded interpretation of background bone turnover markers in response to the intervention. For example, in peri- and post-menopausal females, reduction in inhibition of osteoclastogenesis by osteoprotegerin (OPG), a decoy competitor to the ligand of receptor activator of nuclear factor kappa beta (RANKL), leads to greater bone resorption as a result of RANK/RANKL/OPG dysregulation as oestrogen levels decline (Faienza et al., 2019). It is therefore proposed to apply diverse HIIT at the same duration exclusively in premenopausal females, in order to limit the effect of differences in background oestrogen status on biomarkers of bone turnover.

After demonstrating feasibility of diverse HIIT, acceleration profile and physiological responses to a single bout of training were examined in a female athlete population and sedentary premenopausal females (Chapter 6). Analysis of locomotor data showed that duration of accelerations was greatest in the vertical direction, which suggests that the aim of prescribing impact through vertical landing and take-off actions was successful. However, this study did not investigate the relationship between acceleration profile and background bone biomarker status, nor the effects of chronic training, and ramping of diverse HIIT, on accelerations and markers of bone metabolism.

To address these limitations, the present study investigated the effects of 12 weeks of diverse HIIT in premenopausal women on bone turnover (blood markers and calcaneal bone stiffness), central adiposity and inflammation, in conjunction with characterising acceleration profile.

7.2 Methods

7.2.1 Study design

This study was a randomised controlled training intervention in sedentary females, which aimed to recruit 18 participants to each arm of the intervention.

The primary research outcome under investigation within the group allocated to diverse HIIT was the effect of 12 weeks training on acceleration profile. For one-way within-subjects analysis of variance (ANOVA), with three levels of repeated measures (sampling of accelerations), a power calculation a priori indicated that 18 participants would give effect size f = 0.287 to detect a change in accelerations, with 80% power and α set at 0.05.

7.2.2 Recruitment and allocation

Sedentary women (*n* = 35) in the south west of England were recruited to the study by poster campaign, leaflet drop, circular email communication (monthly University of Exeter staff bulletin) and newspaper advertisement. An initial telephone interview was conducted with women who responded to the recruitment campaign, in which the aims, procedures and training schedule of the study were explained.

Written, informed consent was obtained from women who agreed to participate and met the following inclusion criteria:

- aged 18 or older
- regular menstrual cycle
- not pregnant or attempting to become pregnant
- generally healthy
- no known contraindications to varied intensity exercise
- no current musculoskeletal issue affected by varied intensity exercise

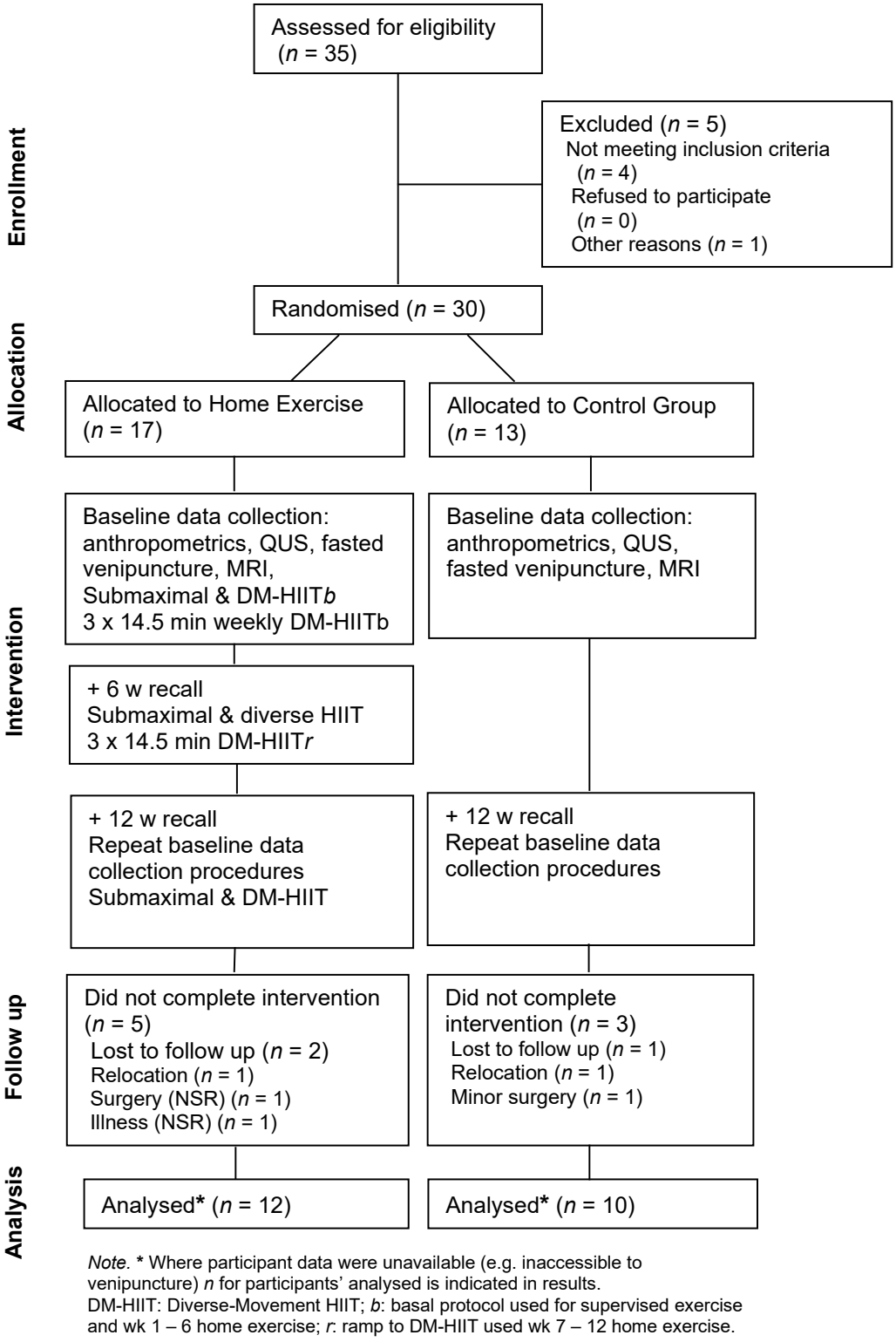
Women were excluded if presenting with one or more of the following criteria:

- undertaking regular exercise above ‘low’ – ‘moderate’ levels
- contraindications to MRI
- taking or having taken medications known to affect bone metabolism
- history of back pain or movement-limiting injury in the previous 6 months

The International Physical Activity Questionnaire (IPAQ) Long Form (August 2002) (http://www.ipaq.ki.se; see Appendix A1.5) was used to evaluate activity status, participants undertaking regular exercise above 'low' –'moderate' levels (IPAQ categorical score 1 - 2) were excluded from the study. Recruitment and allocation procedures are summarised below (Figure 19). Randomisation was performed using a random number generator programme, with numbers previously assigned to each group (Control: 1 – 15; DVD: 16 - 30).

7.2.3 Schedule for data collection

Data were collected at baseline and after 12 weeks, as described in sections 7.2.4 to 7.2.9 (all participants); women allocated to training exercised at baseline, after 6, and after 12 weeks home exercise, during quantified bouts in the laboratory, and data collected as detailed in sections 7.2.10 and 7.2.11.



Note. * Where participant data were unavailable (e.g. inaccessible to venipuncture) *n* for participants' analysed is indicated in results.
DM-HIIT: Diverse-Movement HIIT; *b*: basal protocol used for supervised exercise and wk 1 – 6 home exercise; *r*: ramp to DM-HIIT used wk 7 – 12 home exercise.

Figure 19 Flow Diagram: Diverse HIIT Home Exercise Study

7.2.4 Anthropometric data collection

Participants were asked to refrain from alcohol consumption and exercise activities for 48 hours and to arrive at 8 a.m. in a fasted state (water only after midnight) for baseline and 12 week data collection. Body weight (kg) and height (m) were measured (see General Methods 3.2.1 and 3.2.2) and BMI calculated from an average of three consecutive measurements (Table 21).

Waist and hip circumference were measured (see General Methods 3.2.4) and waist to hip ratio calculated (W:H, AU) (Table 21).

7.2.5 Heart rate and blood pressure at rest

Heart rate and blood pressure at rest were measured after participants had sat quietly for ten minutes (see General Methods 3.2.5) (Table 21Table 21 Participant Characteristics at Baseline).

7.2.6 Medical history and dietary status

A questionnaire (see Appendix A1.1) was used to gather information about medical history, menstruation status and contraceptive use, and consumption of alcohol and tobacco. Weekly calcium intake was assessed by a questionnaire (see Appendix A1.2) validated for evaluation of dietary calcium (Sebring et al., 2007) (Table 21).

Table 21 Participant Characteristics at Baseline (Mean ±SD)

Characteristic	DM-HIIT (*n* = 12)	CON (*n* = 10)	*p*
Body Weight kg	66.1 ±14.5	73.2 ±19.6	0.343
BMI kg.m^2	23.7 ±5.1	28.3 ±6.5	0.078
HR bpm	70 ±11	71 ±11	0.786
Sys mmHg	116 ±15	117 ±15	0.932
Dia mmHg	70 ±9	74 ±11	0.653
MAP mmHg	86 ±11	90 ±11	0.715
Waist cm	78.8 ±12.9	88.3 ±19.9	0.264
Hip cm	94.6 ±12.1	104.7 ±17.2	0.208
Waist to Hip AU	0.82 ±0.05	0.84 ±0.06	0.444
Ca mg.d^{-1}	1611 ±682	1363 ±743	0.437

DM-HIIT: Home Exercise Group; CON: Controls; Sys: Systolic; Dia: Diastolic; AU: Arbitrary Units; Ca mg.d^{-1}: daily Calcium intake.
Note. Baseline data for participants who completed 12 weeks.

7.2.7 Quantitative ultrasound of the calcaneus

A calcaneal scan (GE Lunar Achilles Insight, Bedford, UK) was performed on the left and right calcaneus, as described in General Methods, 3.2.8.

7.2.8 Venipuncture

After measurement of heart rate and blood pressure, venipuncture was performed (see General Methods 3.3.1); blood samples were drawn into yellow top serum vacutainers and handled as previously described (General Methods 3.3.1 paragraph 4) and serum samples were prepared for storage at -80 °C for subsequent analysis (see General Methods 3.3.2).

7.2.9 Analysis of biomarkers

Markers of bone formation and resorption

Serum concentrations of C-Terminal Telopeptide of Type-1 Collagen (β isoform: β-CTXs) (CTX-1) and N-Terminal Propeptide of Type-1 Procollagen (PINP) were measured using electrochemiluminesence immunoassay (ECLIA) on a Cobas e601 analyser (Roche Diagnostics, Germany). The inter-assay CoV

for β-CTXs was ≤3% between 0.2 and 1.5 $\mu g.L^{-1}$ with the sensitivity of 0.01 $\mu g.L^{-1}$. P1NP inter-assay CoV was ≤3% between 20-600 $\mu g.L^{-1}$ with the sensitivity of 8 μgL^{-1}.

C-Reactive Protein (CRP)

Concentration of serum C-Reactive Protein (CRP) was measured by immunoturbidimetric assay on a Cobas e601 analyser (Roche Diagnostics, Germany); 3.1 % for CoV and 6.1 $mg.L^{-1}$ for measure of uncertainty were reported by the laboratory undertaking analysis.

7.2.10 Procedures for interventional exercise

Preparation

Laboratory testing Each participant marked out their exercise area using adhesive tape to form the base and left-hand edges of the floor grid. Participants were instructed to tandem walk (heel of one foot placed immediately after toes of the other) for 12 foot-lengths from the baseline along the left-hand grid line, and an experimenter placed a mark at the end of the 12th step on the floor. Turning right through 90° participants performed a further 12 tandem steps forward and after the 12th step a second mark was made to denote the right-hand top corner of the grid. Turning 90° towards the baseline, a final 12 tandem steps were performed to complete the grid and, after the experimenter had adjusted the right-hand grid line to ensure perpendicularity with baseline and top edges, tape was used to mark out the sides of the grid on the laboratory floor.

Home exercise Participants were instructed to repeat the tandem walking procedure and to mark out exercise area using adhesive tape at the corners, and intermittently along the edges, to represent the floor grid.

Submaximal exercise

Before exercise, participants were fitted with a heart rate monitor (Polar T31, Polar, Finland) and a movement sensor unit (OptimEyeS5, Catapult Sports, Australia) worn in a close-fitting vest supplied by the manufacturer (see General Methods 3.5.1). Participants were instructed to stand still for 10 s after the unit was switched on and then proceeded to a bout of submaximal exercise (see General Methods 3.7 paragraph 2).

Supervised Diverse-Movement HIIT (DM-HIIT)

Immediately after submaximal exercise, participants performed the following diverse HIIT protocol: 480 s (8 x 60 s bouts) of dynamic striding, bounding and hopping, progressing from 30 s 'low intensity' (LI), to 20 s 'medium intensity' (MI) and ending the bout with 10 s 'high intensity' effort, according to the 10-20-30 principle of self-regulated intensity (Gunnarsson & Bangsbo, 2012).

Direction-specific emphasis for actions prescribed during 60 s dynamic bouts was as follows: sagittal- (bouts 1, 2, 6 & 8), frontal- (bouts 3, 4 & 7) and multi-planar (bout 5). Bouts 1 – 5 were followed by 60 s static balance and mobility challenges, at a work to active recovery ratio (W:AR) of 1:1; AR was reduced after bouts 7 and 8 to 30 s, yielding W:AR of 1:0.5. (See Appendix A3.2 for link to presentation of protocol).

Immediately after exercise, the motion sensor unit was switched off.

Unsupervised exercise: diverse HIIT home training

Weeks 1 - 6 Participants exercised at home 3 x per week with the protocol used for supervised DM-HIIT under laboratory conditions. A minimum of 24 hours was advised between home training sessions, with 48 h preferred between sessions, participant schedule permitting.

Weeks 7 - 12 Participants were given a DVD and sent a link to an streamed video to familiarise them with the exercise ramp. Whilst duration and directional emphasis of dynamic 60 s bouts were retained for the 7 – 12 w ramp and W:AR were scheduled as for weeks 1 - 6, increased flight time and shorter ground contacts were presented in the DVD and verbally coached in the soundtrack, to encourage increased height during jumping and bounding. Exercise actions were demonstrated at increased speed, particularly for actions traversing the floor grid and for COD involving decelerations and re-accelerations at its edges. In addition, stiffer-legged landing strategies were coached during landing actions. (See Appendix A3.2 for link to view protocol).

Exercise duration

In total, including transitions between bouts of actions, 860 s of diverse exercise were prescribed. To ensure consistency between laboratory test

conditions and home exercise, participants were asked to repeat at home the procedure used in the laboratory to standardise the exercise area according to multiples of participants' foot length.

Advice to participants

Participants were advised to exercise in the same shoes and on a consistent surface during DM-HIIT home exercise, and recommended to avoid exercising immediately after consuming food, or if in a fatigued state or otherwise unwell. Participants were instructed to contact the researchers if an adverse event occurred associated with home training or if they had a question about any aspect of DM-HIIT administration.

7.2.11 Perception of exercise effort

Participants rated exercise effort using a visual scale from 0 ('rest') to 10 ('maximal'), as described in General Methods 3.6.1, and recorded dated responses in a home exercise log.

7.2.12 Acceleration profile

Participant data acquired during quantified submaximal and DM-HIIT exercise at 0-, 6- and 12 w were downloaded (Catapult Sprint 5.1.7, Catapult Sports, Australia), split into two data files, corresponding to submaximal and DM-HIIT bouts, and exported from the manufacturer's download environment to permit further data analysis, as described in General Methods 3.5.3. Acceleration bands for durations of accelerations during submaximal exercise and DM-HIIT were characterised as summarised in Chapter 6, Table 15.

7.2.13 Statistical approach

A statistical software package (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) was used to perform separate Two-Way Mixed-Model Analysis of Variance (ANOVA) on serum biomarker, QUS and anthropometric data, and blood pressure and HR at rest, with 'intervention group' as the between-group factor (two levels), and 'time' (0 w and 12 w) as the within-group factor.

Linear regression was rejected as an approach to examining the relationship between calcaneal QUS values and body weight, after initial exploration of the data, due to low participant numbers in each intervention

group and inspection of data plots. Therefore, correlation was used, with and without normalisation of parameters to participant body mass, to explore potential associations between these variables.

In the home exercise group, One-Way Analysis of Variance (ANOVA) was used to compare exercise HR variables during quantified submaximal and DM-HIIT in the laboratory at 0-, 6- and 12 w, with 'time' as the three-level repeated measure. For linear and angular acceleration data acquired during DM-HIIT under supervision, separate Three-Way Repeated Measures ANOVAs were used to examine the effect of 'time' (three levels) on the interaction between direction (orthogonal [linear data]; rotational plane [angular data]) (three-level factor) and acceleration band (five-level factor). Where a significant Three-Way interaction was found, separate Two-Way Repeated Measures ANOVAs were used post hoc to examine the effect of training on duration of acceleration within each orthogonal according to amplitude (time [three levels]*acceleration band [five levels)] and the direction-specific effect of training within each acceleration band (time [three levels]*orthogonal/plane [three levels]).

For all analyses, significance was set at $p < 0.05$ and, where used in post hoc comparison, two-tailed T-tests, with Bonferroni correction, were applied.

7.3 Results

7.3.1 Compliance, persistence and adverse events

Thirty five women were recruited and randomised; in total 30 women (n = 12 home exercise [DM-HIIT]; n = 10 controls [CON]) completed the intervention. In CON three participants were lost to follow up, one due to unexpected minor surgery, one due to relocation and another was not contactable for retesting at 12 weeks. In DM-HIIT one participant withdrew due to a non-study related illness, one required surgery that was not anticipated and not related to the study and one participant relocated; two other participants after baseline testing were not able to be contacted for retesting at six weeks (see Figure 19). Twelve participants in the home exercise group completed the 12 week intervention and complied with three self-directed training sessions per week (100% adherence); there were no adverse effects associated with DM-HIIT training, one participant

reported an episode of ankle discomfort and two minor episodes of back discomfort that she did not attribute to training. The participant was given advice, based on adjusting DM-HIIT exercise content with the aim of alleviating reported symptoms, and spontaneous resolution of the issues was reported.

7.3.2 Anthropometric data

No effect of 12 weeks' home exercise was observed for body weight (BW), BMI and hip and waist circumference (Table 22), however BW in CON tended to be greater by 4.3 ±2.4 kg compared with DM-HIIT ($p = 0.094$, $\eta^2 = 0.134$, main effect of group) and there was a tendency for waist to hip ratio to be increased in CON after 12 weeks compared with DM-HIIT ($p = 0.057$, $\eta^2 = 0.186$, time*group interaction effect).

7.3.3 Heart rate and blood pressure at rest

No differences between groups were observed after the intervention ($p > 0.05$ time*group interaction effect) and there were no statistically significant changes in resting heart rate or blood pressure compared with baseline in either group (Table 22).

Table 22 Heart Rate, Blood Pressure and Anthropometry Pre- and Post- 12 weeks (Mean ±SD)

	DM-HIIT (*n* = 12)		CON (*n* = 10)		Interaction	
Characteristic	0 w	12 w	0 w	12 w	*p*	η^2
BW kg	66.1 ±14.5	66.9 ±15.3	73.2 ±19.6	73.2 ±19.7	0.430	0.031
BMI kg.m^2	23.7 ±5.1	24.1 ±5.0	28.3 ±6.5	28.1 ±6.5	0.200	0.081
HR bpm	70 ±11	67 ±7	71 ±11	71 ±11	0.437	0.036
Sys mmHg	116 ±15	113 ±16	117 ±15	119 ±21	0.349	0.049
Dia mmHg	70 ±9	70 ±11	74 ±11	76 ±9	0.641	0.013
MAP mmHg	86 ±11	84 ±12	90 ±11	92 ±12	0.467	0.032
Waist cm	78.8 ±12.9	78.0 ±12.5	88.3 ±19.9	89.2 ±17.9	0.316	0.053
Hip cm	94.6 ±12.1	94.0 ±12.0	104.7 ±17.2	102.7 ±17.7	0.434	0.034
W:H AU	0.82 ±0.05	0.81 ±0.04	0.84 ±0.06	0.87 ±0.04	0.057	0.186

DM-HIIT: Home Exercise; CON: Controls; BW: Body Weight; BMI: Body Mass Index; Sys: Systolic Blood Pressure (BP); Dia: Diastolic BP; MAP: Mean Arterial Pressure; W:H: Waist to Hip Ratio; AU: Arbitrary Units.

7.3.4 Visceral and subcutaneous adipose tissue

Baseline data were not statistically different between groups and therefore were pooled to examine relationships with anthropometric data; volumetric and areal visceral and subcutaneous adipose tissue (VAT and SAT), at the sites measured, were found to be highly positively correlated ($p < 0.001$) with both BMI and body mass (Table 24).

Table 23 Relationship Between Adipose Tissue and Body Weight

	BMI kg.m^{-2}			Weight kg		
Parameter	*R*	R^2	*p*	*r*	R^2	*p*
SAT_{AREAL}	0.940	0.884	<0.001	0.940	0.884	<0.001
$SAT_{VOLUMETRIC}$	0.952	0.906	<0.001	0.932	0.869	<0.001
VAT_{AREAL}	0.858	0.735	<0.001	0.844	0.713	<0.001
$VAT_{VOLUMETRIC}$	0.826	0.682	<0.001	0.838	0.702	<0.001

Note. Analyses performed with 21 participants' data (n = 1 CON unsuitable for MRI).
SAT: Subcutaneous Adipose Tissue; VAT: Visceral Adipose Tissue.

The effect of the intervention on SAT and VAT did not differ between groups ($p > 0.05$ time*group; areal, volumetric VAT, and SAT), however SAT profile differed between groups ($p < 0.05$, main effect of condition): mean areal SAT was 139.2 ±62.6 mm^2 lower in DM-HIIT compared with CON ($p = 0.038$, $\eta^2 = 0.206$) and volumetric SAT was 707.3 ±311.6 mm^3 lower ($p = 0.035$, $\eta^2 = 0.213$) (Table 24); no effect of group was observed for either areal or volumetric VAT.

7.3.5 Quantitative ultrasound of the calcaneus

Calcaneal QUS pre- and post- 12 weeks

No difference between groups was observed for the effect of the twelve week intervention on calcaneal QUS parameters ($p > 0.05$ time*group interaction effect, $QUS_{ALLPARAMETERS}$) (Table 25), however after 12 weeks there was a trend for right (R) BUA to be increased ($p = 0.062$, $\eta^2 = 0.180$, main effect of time, R BUA) and left SOS to be reduced ($p = 0.065$, $\eta^2 = 0.176$, main effect of time, L SOS) (Table 25).

Table 24 Comparison of Truncal Adipose Tissue Pre- and Post- 12 weeks (Mean ±SD)

	DM-HIIT (*n* = 12)		CON (*n* = 10)		Interaction	
Characteristic	0 w	12 w	0 w	12 w	*p*	η^2
Subcutaneous						
Single mm^2	179.3 ±121.2	180.1 ±121.7	324.1 ±169.9*	313.7 ±163.9*	0.263	0.066
Volume mm^3	905.2 ±609.9	901.6 ±602.7	1649.2 ±832.2*	1572.2 ±828.6*	0.188	0.089
Visceral						
Single mm^2	53.8 ±52.8	57.7 ±57.5	92.9 ±62.8	99.1 ±70.1	0.745	0.006
Volume mm^3	288.3 ±297.4	274.3 ±280.5	461.2 ±308.0	479.4 ±329.8	0.353	0.046

DM-HIIT: Home Exercise Group; CON: Control; SAT: Subcutaneous Adipose Tissue; VAT: Visceral Adipose Tissue.
* Significant difference CON vs DM-HIIT ($p < 0.05$, main effect of group).

Table 25 Quantitative Ultrasound Pre- and Post- 12 weeks (Mean ±SD)

	DM-HIIT (n = 12)		CON (n = 9)[△]		Interaction		Time		Group	
Parameter	0 w	12 w	0 w	12 w	p	η^2	p	η^2	p	η^2
SI L AU	92 ±11	95 ±13	98 ±16	94 ±12	0.087	0.154	0.843	0.002	0.382	0.040
SI R AU	93 ±9	95 ±10	95 ±16	97 ±14	0.879	0.001	0.097	0.138	0.480	0.027
BUA L dbMHz.$^{-1}$	103 ±11	109 ±14	112 ±14	111 ±10	0.199	0.095	0.240	0.076	0.402	0.039
BUA R dbMHz.$^{-1}$	102 ±7	105 ±9	111 ±18	116 ±19	0.816	0.003	0.062	0.180	0.310	0.057
SOS L m.s^{-1}	1586 ±24	1582 ±22	1584 ±31	1571 ±28	0.337	0.054	0.065	0.176	0.751	0.006
SOS R m.s^{-1}	1589 ±21	1588 ±23	1573 ±25	1569 ±23	0.839	0.003	0.397	0.040	0.884	0.001
LSI vs RSI $_{ABSOLUTE}$	7 ±9	5 ±4	8 ±7	8 ±7	0.693	0.008	0.586	0.016	0.318	0.052

Note. [△] n = 1 CON 12 week data not collected due to scanner issue.

DM-HIIT: Home Exercise; CON: Control; SI: Stiffness Index; BUA: Broadband Ultrasound Attenuation; SOS: Speed Of Sound

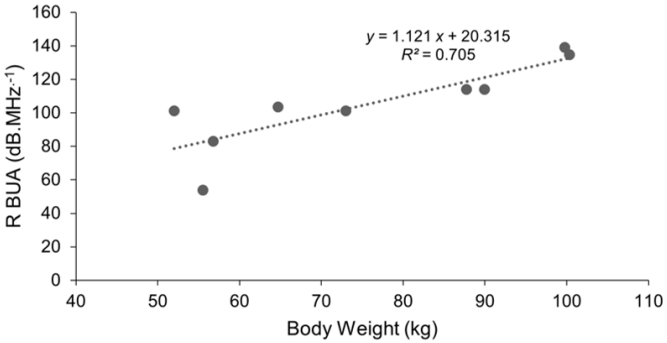
Calcaneal stiffness, body weight and central adiposity

At baseline, body weight (BW) was highly positively correlated with waist circumference (WC) in DM-HIIT (r = 0.92, p < 0.001) and in CON (r = 0.88, p = 0.004) and was also positively correlated with VAT in both groups (r = 0.80, p = 0.002; r = 0.88, p = 0.004; BW versus VAT; DM-HIIT and CON, respectively).

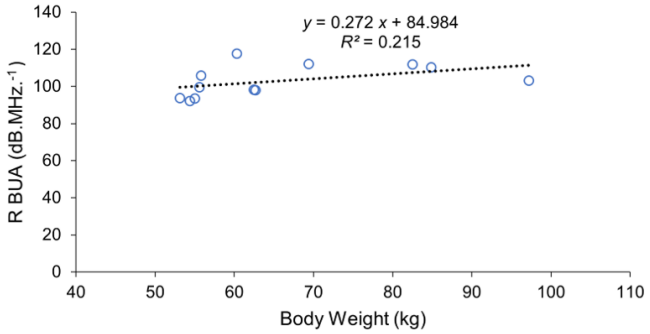
There was also a positive relationship between BW and right calcaneal BUA (R BUA) in CON (r = 0.84, p = 0.005), but not in DM-HIIT (r = 0.46, p = 0.129) (Figure 20), and R BUA was also positively correlated with both VAT (r = 0.91, p = 0.002) and WC (r = 0.81, p = 0.014) in CON, but not in DM-HIIT. However, in CON at baseline, BW was 4.3 ±2.4 kg higher than in DVD (p = 0.094) and both areal and volumetric SAT were significantly lower in DM-HIIT than in CON (p < 0.05) (Table 24).

Adjusting R BUA for body mass (R BUA_{NORM}) a strongly negative relationship was found for both VAT (r = -0.80, p = 0.002) and WC (r = -0.93, p < 0.001) in DM-HIIT, but not in CON (p > 0.05, R BUA_{NORM} versus WC and VAT, in CON) (Figure 20).

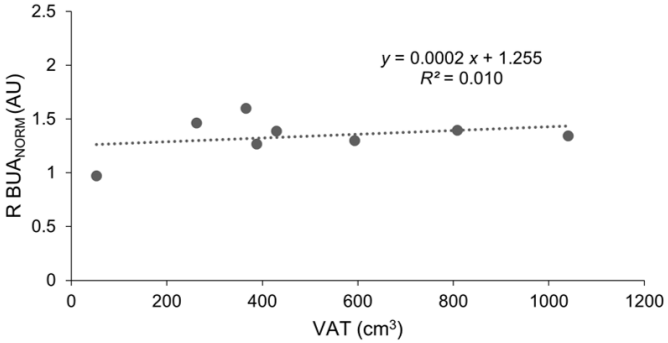
No difference between groups in calcaneal BUA, adjusted for body mass, was observed after the intervention (p > 0.05 time*group interaction effect, R BUA_{NORM}; L BUA_{NORM}).



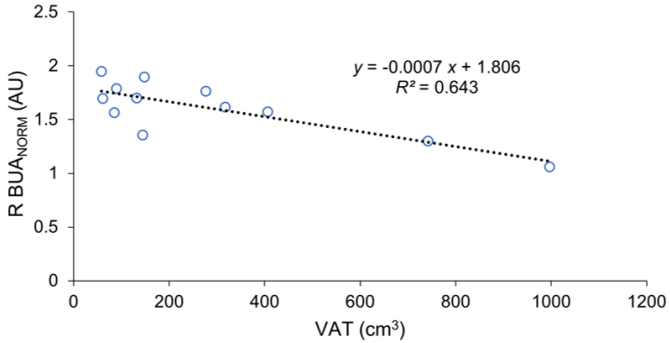
a. Body Weight versus R BUA (0 w) CONTROL



b. Body Weight versus R BUA (0 w) DM-HIIT



c. VAT versus R BUA Normalised to BW (0 w) CONTROL



d. VAT versus R BUA Normalised to BW (0 w) DM-HIIT

BUA: Broadband Ultrasound Attenuation; VAT: Visceral Adipose Tissue.

Figure 20 Calcaneal Stiffness, Body Weight and Visceral Adiposity Relationships at Baseline

7.3.6 Serum biomarkers of bone turnover

There was no difference between groups in the effect of the intervention on background serum P1NP and CTX-1 and no main effect of time for either marker of bone turnover ($p > 0.05$) (Table 26).

Table 26 Serum Biomarkers of Bone Turnover (Mean ±SD)

	DM-HIIT (*n* = 12)		CON (*n* = 10)		Interaction	
Marker	0 w	12 w	0 w	12 w	*p*	η^2
P1NP ug.L^{-1}	46.9 ±18.8	50.7 ±30.8,	40.1 ±6.6	39.8 ±8.7	0.666	0.010
CTX-1 ug.L^{-1}	0.32 ±0.19	0.35 ±0.21	0.26 ±0.09	0.29 ±0.14	0.954	<0.001

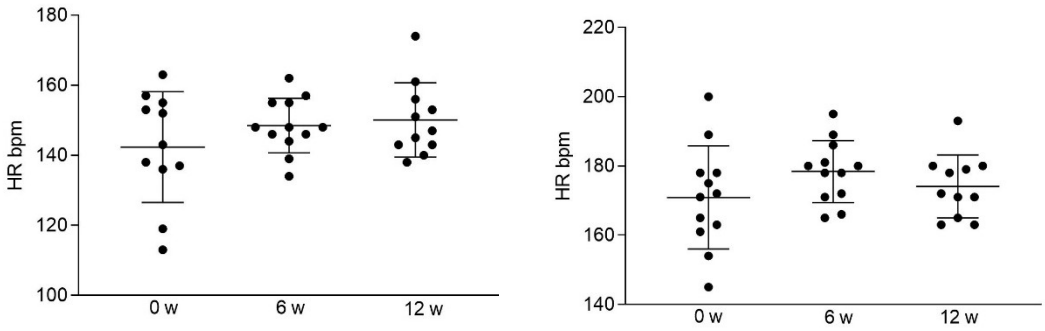
DM-HIIT: Home Exercise; CON: Control; P1NP: N-Terminal Propeptide of Type-1 Procollagen; CTX-1: C-Terminal Telopeptide of Type-I Collagen (β isoform).

7.3.7 Heart rate during diverse HIIT

Training intensity, estimated by mean exercise heart rate (HR) as a percentage of peak HR during quantified DM-HIIT, tended to increase during supervised DM-HIIT as an effect of 12 weeks' home exercise training (p = 0.051, η^2 = 0.282) (Table 27), mean and peak exercise HR did not change (Figure 21).

Table 27 Heart Rate During Supervised DM-HIIT (Mean ±SD)

	DM-HIIT (*n* = 12)			One-way RM Anova	
Parameter	0 w	6 w	12 w	*p*	η^2
HR_{MEAN} bpm	145 ±15	150 ±7	150 ±11	0.177	0.175
HR_{MAX} bpm	173 ±13	180 ±8	174 ±9	0.149	0.173
HR_{MEAN}/HR_{MAX} %	83 ±6	84 ±4	86 ±3	0.051	0.282



a. Mean heart rate during DM-HIIT

b. Peak heart rate during DM-HIIT

Figure 21 Heart Rate During Supervised DM-HIIT (Mean ±SD)

7.3.8 Submaximal exercise

No significant effect of 6 w or 12 w unsupervised DM-HIIT was found for mean or peak HR during submaximal exercise immediately before quantified DM-HIIT ($p > 0.05$) (Table 28).

Table 28 Heart Rate During Submaximal Exercise (Mean ±SD)

	DM-HIIT (n = 12)			One-way RM Anova	
Parameter	0 w	6 w	12 w	p	η^2
HR_{MEAN} bpm	130 ±21	129 ±10	125 ±8	0.690	0.052
HR_{MAX} bpm	158 23	156 ±15	150 ±8	0.612	0.068

RM: Repeated Measures

7.3.9 Perception of exercise effort

No difference was found comparing participants' self-reported RPE scores for weeks 1 - 6 of DM-HIIT home training with scores for weeks 6 -12 (p = 0.425, comparison mean RPE, pre-, post- 6 w ramp). However, RPE immediately after accompanied DM-HIIT at 6 w significantly increased by 1.7 ±0.5 AU (p = 0.013), from 6.2 ±1.4 AU (average of previous 18 unaccompanied sessions) to 7.9 ±1.4 AU, and after 12 w RPE score was 7.9 ±2.2 immediately after accompanied DM-HIIT, compared with 6.9 ±1.9 AU (mean of previous 18 home sessions), a mean increase of 1.0 ±0.7 AU (p = 0.005) above RPE for unsupervised training.

7.3.10 Locomotor profile during supervised DM-HIIT

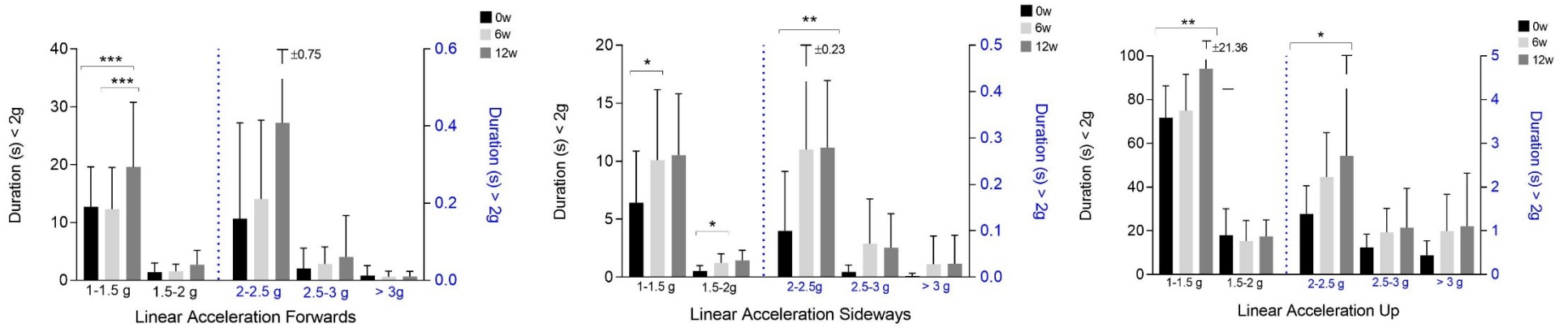
Linear accelerations

A significant effect of home training was observed for linear acceleration profile in the home exercise group (p = 0.019, η^2 = 0.273, training*orthogonal*g band) that was both direction- (p = 0.008, η^2 = 0.262, training*orthogonal) and magnitude- (p = 0.002, η^2 = 0.438, training*g band) specific. Compared with the untrained state, in the vertical direction duration of accelerations increased by 22.4 ±4.1 s between 1 and 1.5 g after 12 weeks DM-HIIT training (p < 0.001), pairwise comparison revealed an increase of 3.3 ±5.5 s for vertical accelerations in this g band after 6 weeks DM-HIIT (p > 0.999) and an increase of 19.2 ±7.3 s after 12 weeks DM-HIIT compared with results after 6 weeks (p = 0.074), suggesting that change was greatest in the vertical direction after the 6 week ramp and when 12 weeks DM-HIIT had been completed (Figure 22).

Lateral accelerations increased by 3.7 ±1.2 s between 1 and 1.5 g after 6 weeks training (p = 0.040), but were not significantly increased after 12 weeks (p > 0.999, 6- versus 12 weeks) and after 6 weeks also increased between 1.5 – 2 g (p = 0.041) with no further significant increase after 12 weeks (p > 0.05, 12 weeks versus 6 weeks). At higher threshold (2 – 2.5 g) lateral accelerations also tended to be increased in duration after 6 weeks (p = 0.088) and were significantly longer than at baseline after 12 weeks (p = 0.005). In the forwards direction between 1 – 1.5 g, duration of accelerations increased by 7.3 ±1.3 s after 12 weeks compared with after 6 weeks (p < 0.001) and was significantly greater than at baseline (p < 0.001) (Figure 22). There was an effect of training on movement characteristics in the frontal plane: duration of lateral accelerations increased significantly between 1.5 and 2 g by 0.7 ±0.2 s after 6 weeks training (p = 0.040) compared with the untrained state, with no evidence of significant adaptation after the DM-HIIT ramp (p = 0.866) (Figure 22). Training exerted an effect on total duration of accelerations during supervised DM-HIIT (p = 0.004, η^2 = 0.396; main effect of training), which increased by 2.5 ±0.5 s after 12 weeks (p = 0.001).

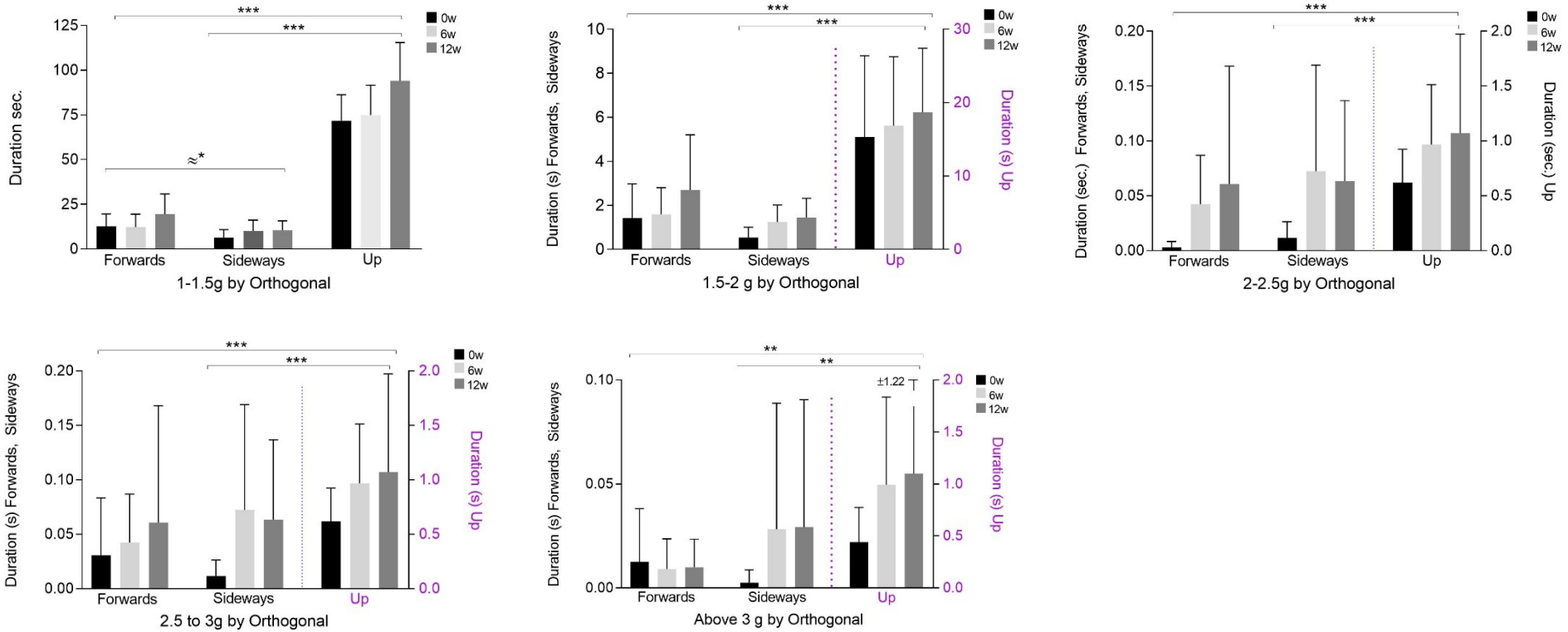
At the highest amplitude of accelerations (above 3 g), a tendency for acceleration profile to reflect training state was observed (p = 0.080, η^2 = 0.206, main effect of condition).

DM-HIIT was characterised globally by non-uniform (p < 001, η^2 = 0.960, main effect of g band) and diverse (p <0.001, η^2 = 0.976, main effect of orthogonal) linear accelerations. At every g band significantly more vertical accelerations were performed than for other orthogonals, and duration of accelerations was greatest between 1 and 1.5 g for all orthogonals (p < 0.001, pairwise comparison between g bands, within orthogonal) (Figure 23). As amplitude of acceleration increased, duration of accelerations significantly decreased compared with the g band immediately below (p < 0.05, pairwise comparison between g bands), except for accelerations in the vertical direction for bands above 2 g, where duration of accelerations did not significantly differ between g bands (p > 0.05, all pairwise comparisons) (Figure 22).



***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$; difference between duration of accelerations.

Figure 22 Comparison of Duration of Linear Accelerations During DM-HIIT Within Orthogonal (Mean ±SD)



*** $p < 0.001$ ** $p < 0.01$; significant effect of orthogonal on duration; ≈* Tendency for a training state*orthogonal interaction effect ($p = 0.052$).

Figure 23 Comparison of Duration of Linear Accelerations Within Acceleration Bands (Mean ±SD)

Angular accelerations

There was a significant effect of training on the interaction between angular acceleration band and plane of motion (p = 0.004, η^2 = 0.264, training*plane of trunk rotational motion*angular acceleration band). Duration of angular trunk accelerations during DM-HIIT differed significantly between the three planes of rotation (p < 0.001, η^2 = 0.608, main effect of angular plane), and differed in duration significantly between angular bands (Rbands) (p < 0.001, η^2 = 0.996, main effect of Rband) and was different between 0-, 6- and 12- weeks' training (p < 0.001, η^2 = 0.646, main effect of training). Therefore, according to the approach used for linear acceleration data, separate two-way repeated measures ANOVAs were used to evaluate the effect of training within each plane of motion (compared effect by acceleration band), and at each level of angular acceleration (compare effect on the duration of axial [left-right], sagittal [flexion-extension] and frontal [side-bending] trunk rotations).

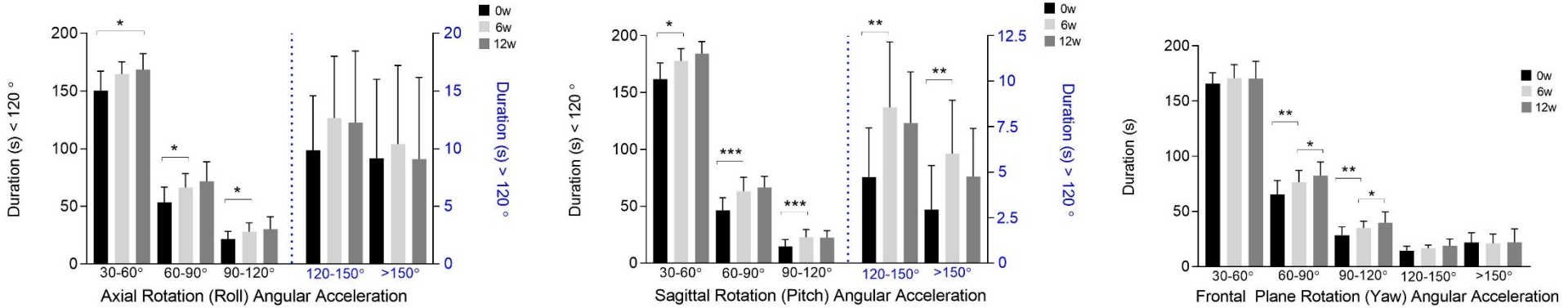
Compared with baseline, duration of axial (left-right) trunk rotation increased (p < 0.001, η^2 = 0.377, training*Rband, within 'roll'), by 18.5 ±5.8 s in the lowest band (30 to 60 Rband) after 12 weeks (p = 0.025, 0 versus 12 weeks), and after 6 weeks by 18.5 ±5.8 s in the 60 to 90 Rband (p = 0.019) and rotational accelerations also increased by 6.0 ±1.9 s in the 90 to 120 Rband after 6 weeks compared with baseline (p = 0.026) (Figure 24 a). No significant changes in duration of axial (left-right) rotations were observed after 12 weeks training compared with after 6 weeks (p > 0.05, all pairwise comparisons, 6- versus 12 weeks).

The largest training effect was observed in sagittal (flexion-extension) trunk accelerations (p < 0.001, η^2 = 0.538, training*Rband, within 'pitch'), which increased in duration within each band after six weeks DM-HIIT compared with the untrained state (p < 0.05, 0 weeks versus 6 weeks, pairwise comparisons within Rband) (Figure 24 b), but no further significant changes were observed after the training ramp (p > 0.05, 6- versus 12- weeks, within Rband).

In the frontal plane, training state exerted a significant effect on angular accelerations (p < 0.001, η^2 = 0.273, training*Rband, within 'yaw'), which was different according to acceleration band and training state, compared with the other two rotational planes. Unlike results for axial and sagittal rotation, trunk side-bending accelerations did not increase in the lowest band with training at

($p > 0.05$, all training state comparisons, 30 to 60 Rband) (Figure 24, c). However after six weeks 11.0 ±2.6 s more accelerations were performed between 60 and 90 degrees.s^{-2} ($p = 0.005$), and 6.5 ±1.7 s more between 90 and 120 degrees.s^{-2} ($p = 0.007$), with further significant increases at these amplitudes after the six-week exercise ramp ($p < 0.05$, 6 versus 12 weeks, pairwise comparisons) (Figure 24 c), suggesting that a training effect of DM-HIIT on frontal plane motion characteristics persisted throughout the 12 week intervention, and was observable at higher bands angular rotation than in the axial and sagittal planes.

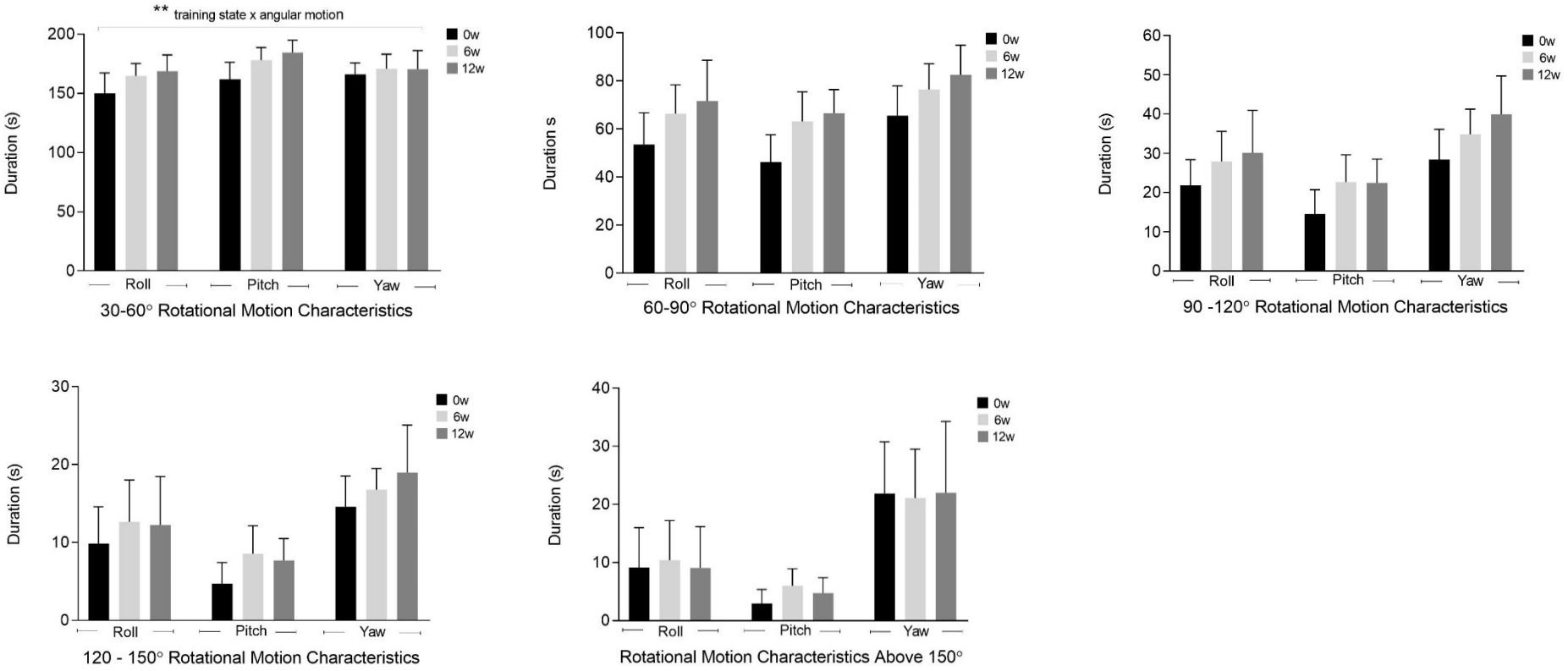
Training state exerted a significant, direction specific effect on duration of accelerations between 30 – 60 degrees.s^{-2} ($p < 0.001$, $\eta^2 = 0.382$, training*angular plane, 30 to 60 Rband) and pairwise comparison showed sagittal accelerations exceeded axial by 13.6 ±3.4 s ($p = 0.007$, pairwise comparison ‘roll’ versus ‘pitch’). There was no other significant interaction between training state and direction of trunk angular rotation within the other Rbands ($p > 0.05$, training*angular plane, above 30 to 60 degrees.s^{-2}).



a. b. c.

** $p < 0.01$; * $p < 0.05$; significant in duration of accelerations.

Figure 24 Effect of Training on Duration of Angular Accelerations Within Orthogonal (Mean ±SD)



** $p < 0.01$; significant interaction between training state and plane of angular acceleration on duration.

Figure 25 Duration of Angular Accelerations Within Angular Band (Mean ±SD)

7.3.11 Locomotor profile during submaximal exercise

Linear accelerations

There was no significant effect of training on the interaction between orthogonal and threshold described by g band (p = 0.464, η^2 = 0.069, training*orthogonal*g band).

Angular accelerations

Training did not exert an effect on angular acceleration profile, characterised by the interaction between trunk rotational plane and angular acceleration band (p = 0.464, η^2 = 0.069, training*angular plane*Rband).

7.3.12 C-Reactive Protein (CRP)

Comparing fasted serum CRP at baseline and after 12 weeks in DM-HIIT (1.5 ±1.5 $mg.L^{-1}$ versus 1.6 ±1.5 $mg.L^{-1}$) and CON (5.3 ±6.5 $mg.L^{-1}$ versus 3.7 ±5.2 $mg.L^{-1}$) no significant effect of the intervention was found (p = 0.308, η^2 = 0.55, time*group). CRP tended to be lower (p = 0.075, η^2 = 0.158, main effect of group) by 3.0 ±1.6 $mg.L^{-1}$ in DM-HIIT compared with CON (p = 0.075). Body weight in CON was 4.3 ±2.4 kg heavier than in DM-HIIT (p = 0.094, η^2 = 0.134, main effect of group) and in CON areal and volumetric subcutaneous adipose tissue were significantly higher than in DM-HIIT (p < 0.05) (see 7.3.4).

7.4 Discussion

Twelve weeks' home-training with 3 x weekly bouts of brief (14.5 min) multidirectional HIIT, resulted in a significant training effect on acceleration profile during exercise in sedentary females. Duration of accelerations above 1 g increased in all orthogonals and at every threshold, and were significantly greater in the vertical direction, compared with accelerations at baseline, during a matched bout of diverse exercise.

Enhanced capacity for vertical accelerations was accompanied by an elevation in training intensity, characterised by exercise heart rate, however despite evidence of locomotor and cardiorespiratory adaptation after 36 bouts of training, no change in calcaneal bone, or in background serum biomarkers of bone formation and resorption, were observed in the home-exercise group, compared with controls. Results therefore suggest that whilst 12 weeks' diverse movement (DM)-HIIT elicited a training effect on vertical acceleration capacity, mechanical strains derived from exercise actions did not exert a measurable osteogenic effect, at the interventional duration and training dose applied.

During quantified exercise, a direction-specific effect of chronic home training was observed. Whereas linear accelerations above 1 g increased overall by 41.9 ±8.7 s after 12 weeks, duration of vertical accelerations was selectively, and highly significantly increased by 19.8 ±6.5 s, compared with accelerations forwards, and by 23.0 ±5.5 s compared with accelerations sideways. This selective adaptation in locomotor profile was presumed to derive from enhanced capacity to perform repeated jumping, hopping and lateral bounding, which were prescribed on the basis of duration, rather than number of repetitions, during low- (30 s), medium- (20 s) and high-intensity (10 s) phases of the protocol. Accordingly, the significant increase in vertical accelerations was attributed to participants executing a higher volume of exercise actions involving axial displacement and vertical motion in the trained state.

However, although locomotor profile demonstrated there was a directionally diverse, and predominantly vertical, acceleration response to DM-HIIT, durations of accelerations within each orthogonal were greatest at the lowest threshold categorised (1 - 1.5 g), and above 3 g only tended to be significantly increased after home training, from 0.4 ±0.3 s at baseline to 1.1

±1.2 s. A greater training effect on acceleration characteristics at lower thresholds, within the overall signal describing locomotor responses, could partly explain evidence that dynamics of background bone formation-resorption biomarkers, and calcaneal bone, did not change. As previously discussed, in relationship to acceleration profile during evaluation of the prototype for DM-HIIT (Chapter 6, Section 6.4 paragraphs 7 – 8), it is possible that exposure to high-intensity signal, analogous here to accelerations at > 3 g, was not adequate under conditions of exercise prescription applied here, and therefore did not promote an osteogenic response.

Studies that have examined daily acceleration profile provide evidence that active constituents of the bone anabolic signal, derived from locomotor activity, reside at higher intensity. In postmenopausal women a positive association between brief, daily exposure to HIA, quantified as accelerations above 1.5 g, and tibial periosteal circumference has been reported (Hannam et al., 2017), and increased tibial areal and cortical BMD in male and female adolescents was observed for greater daily HIA, identified as vertical accelerations above 4.3 g (Deere, Sayers, Rittweger, & Tobias, 2012). The difference between thresholds applied to define HIA in these populations suggests that parameter definition for intensity demonstrates relative, rather than absolute, specificity, and this can be explained by evidence elsewhere that anabolic effects of exercise actions are mediated by biological status and body composition, particularly for bone. For example, ablation of endogenous oestrogen after menopause appears to attenuate the anabolic effect of exercise-induced loading in women (Tobias, 2003; Zhao et al., 2017), and reduced skeletal responsiveness to habitual HIA, associated with lower body fat, has been reported in cross-sectional examination of adolescent females (Deere et al., 2012). In the present study, gonadal steroid hormone (GnH) status was not assessed, which could be useful to examine in future interventional application of the protocol, to evaluate the relationship between GnH profile and skeletal responses to DM-HIIT. No history of amenorrhea or menstrual dysfunction was reported by participants in medical questionnaires, however, serum CRP, a marker of inflammation, tended to be higher in controls, and areal and volumetric subcutaneous adipose tissue were significantly higher than in the home exercise group, indicating higher inflammation, in association with pre-obese level BMI (De Lorenzo et al., 2019) in the non-exercising group.

In designing diverse HIIT, it was hypothesised that ground reaction forces, generated by high-intensity actions (HIA) that elicited vertical accelerations and included a flight phase, could provide a stimulus to bone adaptation through landing impacts, if these generated sufficiently unusual and high-magnitude mechanical strains during execution. According to the proposal that supra-normal skeletal loading initiates bone adapaptation (Frost, 1987), mediated by co-operative osteoblast-osteoclast activity within bone (Hinton, Rackard, & Kennedy, 2018; Ozcivici et al., 2010), it may be inferred from present findings that either the stimulus provided by diverse HIIT did not provide sufficient magnitude or versatility of mechanical strain to initiate an osteogenic response, or that investigative methods and procedures were not sufficiently sensitive to detect such an effect. For example, in a clinical setting, bone biomarker responses to antiresorptive treatment can be measured in as little as two weeks, however, 12 -24 months may be required for a meaningful change in BMD (Song, 2017). At 12 weeks, the present intervention was at the lower limit for detection of an anabolic response, although this has been reported, for example after 12 weeks high-impact dance, which resulted in significantly increased total hip and femoral areal BMD in 30 -50 y women (Ubago-Guisado, Sanchez Sanchez, Vila Maldonado, & Gallardo, 2019), and after 14 weeks' recreational football training, which increased volumetric tibial BMD in premenopausal females (Helge et al., 2010).

In fact, the rationale for HIA selected for the home exercise programme drew on evidence of osteogenic effects, attributed to these participatory actions, in recreational team sports (Krustrup, Helge, et al., 2018; Milanović et al., 2019). Therefore, present results indicate that dose characteristics and training context, as well as limitations implicit in the 12 week duration of the study, may have influenced, and possibly attenuated, potential osteotropic outcomes. For example, an increase in BMD, but only at the femoral neck, was observed in young women after 6 months' low-repetition (10 maximal efforts) jumping (Kato et al., 2006), which comprised three weekly training bouts, and in schedule accords with home exercise in the present study. During DM-HIIT, maximal effort vertical jumping was included during a single 10 s phase of high-intensity actions (HIA). Therefore, it may be speculated that whilst DM-HIIT featured an impact activity, demonstrated to elicit site-specific osteogenic adaptation at the same weekly training frequency, duration allocated to HIA was inadequate to

accumulate sufficient exposure to high intensity impacts and promote an anabolic effect. Alternatively, it is possible that muscular efforts incurred during other exercise actions attenuated jump height, and therefore impact magnitude, in accordance with what has been shown during fatiguing vertical jump repetition (Rodacki, Fowler, & Bennett, 2002). Whole-body deceleration, effected by eccentric contractions of hip and lower limb muscles, were imposed at high frequency due to the reduced exercise area, and these actions could have induced fatigue and reduced power during subsequent jumping phases, as demonstrated for exercise in research simulating the work-rate observed during football (Rahnama, Reilly, Lees, & Graham-Smith, 2003). Therefore, scheduling maximal effort jumping early in DM-HIIT, and for longer, could result in a greater volume of higher-magnitude mechanical strain from HIA, and consequently amplify the potential for an osteogenic effect.

Further support for a proposal of potential under-prescription of HIA in the present protocol, is provided by evidence of a dose-response effect of impact in premenopausal females. A study which applied single bouts of 50 unilateral jumps reported 0.9 (-0.1 – 2.0)% increase in femoral neck BMD at a frequency of 4 x weekly, which increased to 1.8 (0.8 – 2.8)% for 7 x weekly (i.e. daily) jumping (Bailey & Brooke-Wavell, 2010), suggesting > 4 weekly bouts may be required to elicit femoral adaptation for jumping titrated at low-volume, as in brief, diverse HIIT in the present study. In total, 195 s of unilateral jumping actions were included during DM-HIIT, consisting of 20 s flighted stepping at low-intensity (LI), 105 s lateral and diagonal progressive bounding at medium-intensity (MI), and 80 s hopping and lateral jumping at high-intensity (HI) (See link to view protocol, Appendix A3.2), with the aim of providing versatile strains that approximated the diverse, multidirectional environment of team sports. Therefore, unlike the approach adopted in previously cited interventions (Bailey & Brooke-Wavell, 2010; Kato et al., 2006), hopping and bilateral jumping were treated here as progressive, rather than stereotypical actions, and incorporated within flighted, multidirectional movements across the floor grid, rather than being executed ‘on the spot’. Jumping, without intention to progress horizontally, is shown to generate a predominantly axial acceleration signal and vertical ground reaction forces (GRF) during landing impacts (Quagliarella, Sasanelli, Belgiovine, Moretti, & Moretti, 2010). It may therefore be proposed that optimising versatility, within the array of mechanical strain signals

generated by exercise, could have compromised the potential of the regimen to elicit higher magnitude vertical impacts, and could account for the low-volume of high-threshold (> 3 g) accelerations observed in locomotor profile. In future prescription, enhancing vertical, rather than progressive, movement bias during flighted actions could amplify the potential for bone anabolic effects of DM-HIIT, by increasing magnitude of ground reaction forces during landing impacts. Furthermore, as the window for detection of bone biomarker responses post-exercise has been shown to be brief (Rogers, Dawson, Wang, Thyfault, & Hinton, 2011), the effect of enhancing vertical acceleration content could be more precisely examined by sampling biomarkers immediately after diverse HIIT, as undertaken after SSG and WBV in the previous study (see Chapter 4, 4.2.6), rather than at rest, as performed here. Whilst care was taken to capture 'true' background biomarker status, by collecting samples at the same time of day and a minimum of 48 h after previous training in the home exercise group, it is possible a transient elevation in bone turnover markers occurred immediately after diverse HIIT that was not characterised. Nevertheless, a cross-over trial in younger (~23 y) women did not observe any change in P1NP and CTX-1 after 8 x 60 s bouts, at a work to rest ratio of 1:1, of either cycling or running (Kouvelioti et al., 2018), which tends to suggest that impact is not an exclusive determinant of acute bone biomarker responses to brief, intense exercise.

It may also be speculated that a localised bone anabolic response to home training could have occurred but was not accessible in participants with calcaneal ultrasound. Previously cited evidence of bone adaptation after low-repetition jumping was observed to be localised at the femoral neck (Kato et al., 2006) and similary, increased BMD, but only at the femoral neck, was reported in another longer-term (18 month) jumping intervention in premenopausal women (Heinonen et al., 2012). As the calcaneus, in common with the vertebral column, is primarly comprised of trabecular bone (> 90 % for the os calcis), and in premenopausal women meta-analysis supports an osteogenic effect at the femur, but not the spine, in response to impact exercise (Zhao et al., 2014), calcaneal QUS may not have resolved an adaptive response to loading, yet this might have been detectable at the femur using DXA. Moreover, sensitivity and specificity of calcaneal QUS indices to predict BMD at the femoral neck have been reported to be only 70% in younger (19 y) women, and 76.8% in middle-aged and older women (Iida et al., 2010), and to be significantly more correlated

with trochanteric cancellous bone than femoral neck BMD (Zhang, Lv, et al., 2015). This suggests parameters assessed by QUS are only moderately representative of bone, in regions demonstrated to exhibit a response to impact, in a similarly aged population to that of the present study.

After results in sedentary women showed diverse HIIT imposed high physiological demands (see Chapter 6, section 6.3.4), the regimen for home exercise was modified. Whilst no adverse events or training-related injuries were reported for the protocol, adaptations designed to increase tolerance, and account for sedentary status at baseline, could have attenuated the potential of exercise to exert an osteogenic effect. For example, three 60 s phases of activity, prescribed previously (see 6.2.8) as 'walk' (30 s), 'jog' (20 s) and 'sprint' (10 s), according to the 10-20-30 concept of ramped intensity (Gunnarsson & Bangsbo, 2012), were replaced during weeks 1 – 6 of home training with 60 s 'walk' (30 s), 'walk fast' (20 s) and 'jog' (10 s), to reduce intensity. In sections featuring coronal plane (i.e. side to side) progressions actions were modified in choreography, and in audio-visual demonstration, to account for deficits in posterior hip and lower limb strength identified in sedentary locomotor profile (Cabell, Pienkowski, Shapiro, & Janura, 2013; Fukuda et al., 2012; Willoughby & Copeland, 2015), and to reduce the risk of adverse events (AE), such as trips and falls, in a domestic setting. Consequently, 60 s lateral bounding and jumping were modified to 60 s side-stepping and low-flight hopping. Reducing speed of execution during these movements, and encouraging longer ground contact time, would decrease the slope of the associated acceleration curve (Heikkinen et al., 2007) and lower the impulse from interaction of body mass and ground reaction forces (GRFs) (Maldonado, Soueres, & Watier, 2018). Similarly, demonstrating a shorter flight phase and lower vertical jump height in choreography for the untrained state, to provide an acceptable training ramp to higher intensity efforts, would be predicted to lower GRFs as reported for softer landing strategies (Guy-Cherry, Alanazi, Miller, Staloch, & Ortiz-Rodriguez, 2018; Myers et al., 2011), which reduce corresponding impact intensity (Clissold, Cronin, De Souza, Wilson, & Winwood, 2019; Puddle & Maulder, 2013), thereby attenuating potential osteotropic signals from exercise impacts during the first ramp.

Acceleration responses to supervised training after 6- and 12- weeks offers some support for this proposition: an increase in duration of vertical and lateral accelerations at higher threshold (2 – 2.5 g) was observed after 12 weeks, but not after 6 weeks, suggesting that a training effect on higher-threshold acceleration capacity was only detected after the full 12 weeks of training. Moreover, the 6 week ramp to home exercise encouraged greater reactivity, briefer ground contacts and increased flight phase during exercise actions. Therefore, it is also possible to attribute the improvement in acceleration capacity after 12 weeks to the exercise ramp providing an effective stimulus to locomotor adaptation at higher thresholds of accelerations. Alternatively, the observed increases in duration of these accelerations could be attributed to greater familiarisation with the choreography and acclimatisation to administration of DM-HIIT, in a previously naïve, inactive cohort.

With respect to the latter proposal, context of training within the present study could also have influenced osteogenic outcome, by acting as a moderator of performance intensity, and could therefore be implicated in physiological responses to exercise. This hypothesis is somewhat supported by rating of exercise demand, which was significantly higher for accompanied DM-HIIT at 6- and 12 weeks, compared with home exercise. It may be speculated from these results that intensity reduced during unsupervised training, which could have lowered the osteogenic potential of actions featured in the protocol, and it is even possible perception of effort was affected by training surface. During supervised DM-HIIT participants exercised on a hard surface, whereas at home the reported tendency was for exercising indoors on carpeted floors. Speculatively, the softer surface used in regular home training could have attenuated magnitude of impact and introduced friction during foot contacts, which are factors demonstrated to affect derivatives of the stress-strain relationship that characterise landing intensity (Nigg & Yeadon, 1987) and thus could have mitigated impact during habitual training. Moreover, in team sports, whilst small-sided games on a yielding surface have been shown to elevate anaerobic demand, impacts from HIA tend to be lower compared with firmer terrain (Brito, Krustrup, & Rebelo, 2012). Nevertheless, despite the potential for confounding effects for exercise in a domestic context, evidence from a sedentary, middle-aged population suggests adherence to high-intensity exercise in a home setting is greater than for medium intensity (MI) (Byrne,

Caulfield, & De Vito, 2018), and this is supported by present results for diverse HIIT, with no participants lost to unacceptability of the protocol and high attendance (100% compliance with 36 sessions).

Furthermore, exercise heart rate (HR) after 12 weeks' home training suggested a progressive elevation in training intensity (HR_{MEAN} / HR_{PEAK} *100), from 83 ±6% at baseline, to 84 ±4% after 6 weeks, and 86 ±3% after 12 weeks and, during the final training bout, exceeded the threshold of 85-95% intensity proposed to define HIIT (Ito, 2019). Alongside the increase observed in exercise HR, there was a highly significant main effect of orthogonals: vertical accelerations above 1 g increased from 89.5 ±6.2 s to 117.7 ±32.1 s, compared with an increase from 14.3 ±2.5 s to 22.8 ±13.4 s forwards, and 7.1 ±1.4 s 12.3 ±6.2 s sideways, during the final training bout. Evidence of a progressive increase in training intensity, alongside improvement in vertical acceleration capacity, suggests that after 12 weeks participants were physiologically adapted to diverse HIIT above basal sedentary status. It may therefore by hypothesised, were the intervention to have continued, that participants were sufficiently diverse-movement trained after 12 weeks to derive benefits to bone, if the observed tendency towards increasing higher-threshold accelerations were sustained.

Whilst in the present study motor competency was not formally examined, it could be argued that pre-acquired skills and movement traits influenced aspects of exercise performance, particularly landing mechanics, which characterise the interaction between gravity, body weight and limb and torso alignment during impact. Evidence that lower limb kinematics influence magnitude and distribution of ground reaction forces during landing and foot contacts (Standing & Maulder, 2015; Zhang, Fu, & Liu, 2019) provides a plausible mechanistic explanation for the positive relationship observed between early motor competency (MC) and bone strength in late adolescence (Ireland, Sayers, Deere, Emond, & Tobias, 2016). Furthermore, as variability in bone strain for a given force has been proposed to reflect anatomical differences between individuals (Troy, Mancuso, Butler, & Johnson, 2018), deploying specific movement coaching in an interventional setting, to investigate the impact on bone outcomes, is worthy of further exploration, particularly as a role for feedback in modulating landing impact forces has been

reported (Ericksen, Gribble, Pfile, & Pietrosimone, 2013). This could be achieved, in future DM-HIIT interventions, by coaching stiffer-legged landing and encouraging ‘slamming’ through the heel into the forefoot on impact at the edge of the grid. These movement strategies have been shown to elicit higher GRFs (Standing & Maulder, 2015) by adjusting lower limb kinematics and increasing impact intensity (Ericksen et al., 2013; Myers et al., 2011; Niu et al., 2014), and therefore could augment the osteotropic signal from diverse HIIT.

7.4.1 Limitations

There were several limitations to the present study: we aimed to recruit 18 participants to each arm and as we did not achieve this, the study was underpowered. Although this study was designed for home exercise, a disadvantage of this is that conditions of training were not rigorously controlled. This could have affected bone outcomes through attenuation of impacts in participants exercising on a carpeted, or other soft indoor surface, in a domestic setting. A further limitation is that acceleration profile was characterised from trunk-worn motion sensor technology, therefore vertical accelerations cannot be ascribed solely to flighted actions which incur ground reaction forces during landing. Accordingly, the training effect observed on vertical accelerations after 12 weeks is likely to represent deceleration and acceleration actions at the edge of the grid, during which the trunk exhibits axial displacements, as well as possibly implying a higher volume of impact from executing more jumping and hopping. Finally, methods used to quantify bone outcomes may not have been adequate to characterise bone responses: calcaneal QUS is only moderately related to femoral bone indices, where adaptation could have occurred in this premenopausal population, and sampling bone biomarkers at rest may not represent bone metabolic profile immediately after exercise.

7.4.2 Conclusion

There was evidence of a training effect on vertical acceleration profile in sedentary premenopausal women after 3 x weekly bouts of brief, diverse HIIT for 12 weeks. Background bone turnover markers and calcaneal QUS indices did not show a measured osteogenic effect of exercise, and the study was underpowerd to detect this. However, locomotor profile suggested modifications to accommodate basal sedentary status and increase tolerance could have

attenuated impacts during the first 6 week phase of the intervention. Further studies are required, for example in an athlete population with presumed higher motor competence, to assess the effect of ramping exercise content and encouraging movement strategies that amplify landing impact, to assess the effect on duration of higher-magnitude accelerations and bone outcomes.

8 Effects of 12 Weeks Supervised Diverse HIIT on Bone Metabolism and Calcaneal QUS in Young Male and Female Ballet Dancers

8.1 Introduction

Dance is an aesthetic performance discipline, and therefore as well as technical and interpretative skills, dancers are often selected for their physical qualities (Walker, Nordin-Bates, & Redding, 2010), particularly students progressing through the stages of classical ballet training (McCormack, Bird, de Medici, Haddad, & Simmonds, 2019). In ballet, gracile, long-limbed physiques tend to be favoured (Claessens, Beunen, Nuyts, Lefevre, & Wellens, 1987), and it has been suggested that this could lead to restrained eating behaviours, in order to maintain the competitive advantage of a lean somatotype (Haight, 1998).

Drive for thinness can serve as a proxy indicator of energy deficiency in exercising women (De Souza, Hontscharuk, Olmsted, Kerr, & Williams, 2007), and even moderate energy imbalance may attenuate gonadotropic hormone (GnH) secretion by disrupting hypothalamic pulsatility (De Souza, Lee, et al., 2007; Loucks & Thuma, 2003). Decreased pulse frequency of luteinising hormone (LH) is considered the initial event in menstrual disturbance (MD) frequently observed in states of low and negative EA (Koltun, De Souza, Scheid, & Williams, 2020; Williams, Helmreich, Parfitt, Caston-Balderrama, & Cameron, 2001). Evidence of metabolic derangement, discerned in reduced circulating levels of triiodothyronine (T_3), insulin-like growth factor 1 (IGF-1), and leptin, suggests that MD represents metabolic repartitioning as an adaptive response to low EA (Williams, Koltun, Strock, & De Souza, 2019). Assessment of eating behaviours (EB) and physical activity (PA) in female contemporary dance students suggests energy intake is unlikely to meet energy demands imposed by full-time practice and performance (Brown, Howatson, Quin, Redding, & Stevenson, 2017), and cross-sectional examination of EB in vocational female ballet students found reduced energy availability (35 – 45 kcal.kg.free fat mass [FFM].d^{-1}) in 44%, and menstrual dysfunction (MD) in 40% (Civil et al., 2019). Dancers may therefore be at risk of subtle and more profound MD associated with imbalance between energy intake and energy

demands of training and performance, and this is supported by evidence of low EA, menstrual irregularity and low BMD in elite female ballet dancers (Doyle-Lucas et al., 2010; Hoch et al., 2011). Prevalence estimates ranging from 10 – 46.5% for low BMD/osteopenia and 8.9 – 23.8% for osteoporosis have been reported in dancers (Hincapié & Cassidy, 2010), however, a predominance for cross-sectional reporting in the available research has limited insight into the evolution of low EA, MD and skeletal endpoints in this population. In one of the few longer-term studies into dancers' musculoskeletal health and MD, amenorrheic dancers were found to have lower whole-body BMD than their eumenorrheic counterparts across all timepoints surveyed (Warren et al., 2002), underscoring the potential for serious consequences to bone health in these athletes, through achievement of lower peak bone mineral density. Moreover, studies investigating EB and MD in dancers have tended to utilise food diaries and rely on self-report, and whilst invaluable to provide insight into nutrition behaviours within the performance milieu, there is a lack of quantitative evidence characterising the relationship between gonadal hormones and systemic indicators of bone metabolism, particularly in ballet dancers, who are a hard to access population.

Whereas the Female Athlete Triad (Triad) has been an adequate schema to describe the inter-related phenomena of disordered eating, menstrual dysfunction and bone impairment in females (STAND, 2007), it is increasingly recognised that male athletes may also experience metabolic and hormonal consequences of low energy availability (Mountjoy et al., 2018). Accordingly, it was proposed that the term Triad should be replaced by Relative Energy Deficiency in Sport (REDS) (Mountjoy et al., 2014), to acknowledge that clinical manifestations of pathological adaptation to EA are not uniquely female.

Whilst the gonadal hormone milieu in males is different, higher prevalence of bone injuries and reduced BMD in sports codes emphasising somatotypical leanness, such as male endurance and weight class athletes (Tenforde, Barrack, Nattiv, & Fredericson, 2016), is indicative that skeletal responses to low EA may be phenomenologically similar to what is observed in REDS in females. This is borne out by evidence in exercising males that metabolic hormones and indicators of energy homeostasis are profoundly disturbed in response to acute induction of low EA (Koehler et al., 2016). In a

longer-term scenario, this raises the possibility that male athletes who are vulnerable to conditions of low and negative EA could experience metabolic dysfunction, and consequent musculoskeletal impairment, within an array of possible maladaptive responses to energy conservation (Elliott-Sale, Tenforde, Parziale, Holtzman, & Ackerman, 2018). For example, compared with controls, male jockeys exhibit reduced femoral neck BMD, low whole-body bone mass and lower levels of bioavailable testosterone (Dolan et al., 2012), which suggests chronic REDS could indeed initiate bone impairment in male, as well as female, athletes in disciplines where leanness confers competitive advantage. However, in a controlled setting, using biomarkers to assess bone metabolic responses to low EA, a catabolic response has not been conclusively demonstrated in males. For example under restricted EA, whereas bone resorption was acutely upregulated and formation downregulated in exercising females, it did not change in males (Papageorgiou et al., 2017). Therefore, both the immediate and longer-term impact of impaired energy availability in male athletes exhibiting eating restraint, or inadvertently failing to match energy requirements of training, are unclear. Furthermore, inaccessibiliy of athletes due to demands of training and competition, and burden on resources and costs, have limited widespread use of dynamic indicators of bone metabolism. Therefore, reference data characterising bone biomarkers in athlete populations, which differ according to somatotypical pressures and training burden, are lacking.

Historically, perhaps associated to earlier identification of the Triad with clinical manifestations of its components in females, research in leanness athltes has been biased towards investigating female athletes. For example, in ballet dancers, investigation has focussed on manifestation of REDS associated dysfunction on bone and gonadotropic hormones (Doyle-Lucas et al., 2010; Valentino et al., 2001; Warren et al., 2003) and characterisation of BMD in females (Amorim et al., 2015; Wewege & Ward, 2018), whereas gonadal hormone (GnH) profile and indicators of bone metabolism in male ballet populations are under-represented. However, a recent study in ballet dancers reported that resting metabolic rate (RMR) was suppressed in males, alongside evidence of both inadvertent and cognitively driven low EA (Staal, Sjodin, Fahrenholtz, Bonnesen, & Melin, 2018). In a cross-sectional study in male and female vocational ballet students, BMD in dancers was lower at both impact and

non-impact sites (Amorim et al., 2017), which may be variously interpreted. It may be inferred that dance practice does not provide a sufficient stimulus to osteogenic adaptation in adolescent athletes but could also indicate potential attenuation of mechanical strains elicited by training in both males and females, speculatively linked to low GnH, and metabolic adaptation to energy conservation, as described. To examine lines of evidence in either scenario, and to extend what is currently described in this population, particularly in males, further investigation is required. Comprehensive description of background GnH status, biomarker indicators of bone metabolism, and their behaviour under conditions of intensive dance training, are not available. Therefore, an interventional approach to characterising these indices could provide further insights into relationships between hormonal milieu, training adaptation and responses in bone. It would also supply more comprehensive reference data, which are currently lacking in dance athletes, as to date small sample sizes are reflected in the literature, due to the specialised nature of the population. Addressing this gap in the evidence could also help inform surveillance in this population, particularly in dancers in the later stages of vocational training, with the aim of optimising dancers' musculoskeletal and metabolic health.

In addition to potential risk of bone impairment from REDS-associated dysfunction, research into ballet dancers' fitness has identified a tendency for these athletes to be insufficiently aerobically conditioned (Koutedakis & Jamurtas, 2004), yet cardiorespiratory demands of dance performance are demonstrated to be high (Wyon, Abt, Redding, Head, & Sharp, 2004). As aesthetic athletes, ballet dancers acquire performance skills through long-term repetition of highly-specific body weight movements, and the inadequacy of usual training to elicit cardiometabolic and strength adaptations could explain the gap identified between practice and performance (Koutedakis & Jamurtas, 2004; Twitchett, Koutedakis, & Wyon, 2009). As time-motion analysis demonstrates dance is an intermittent performance code (Wyon et al., 2011), and therefore highly taxing of both the aerobic and anaerobic energy systems (Bangsbo et al., 2006), lack of alignment between habitual training and elite performance demand could be addressed by supplemental exercise. It has been suggested that alongside regular dance training, bouts of aerobic conditioning could address inadequacies identified in dancers' cardiometabolic

capacity and enhance capacity to perform high-intensity actions under conditions of intermittence (Angioi, Metsios, Koutedakis, & Wyon, 2009). However, an emphasis on cardiometabolic adaptation is not exclusive to providing a stimulus to bone. It may therefore be proposed that supplementing ballet dancers' regimens with exercise designed to be osteogenic, whilst also exceeding cardiometabolic demands of habitual training, represents an ecologically relevant objective for improving dancers' performance fitness and bone health.

We previously demonstrated (Chapter 5, 5.3.1) that a brief, varied intensity protocol featuring team sports activities was acceptable in inactive females, and elicited high training heart rates and blood lactate concentration, during acute exposure in sedentary females and female ballet dancers (Chapter 6, 6.3.4). However, in a 12 week intervention, although vertical accelerations and exercise intensity increased during supervised exercise after 36 training sessions, no change in background biomarkers of bone metabolism or calcaneal bone were observed (Chapter 7, 7.3.5; 7.3.6). It was speculated that this could reflect lower exercise intensity during unsupervised training at home. Furthermore, as these women were not athletes, lack of adaptation to team sports actions, and movement competency in the basal training state, could have attenuated the potential for exercise actions to be osteogenic, and account for the low duration of high threshold (> 3 g) accelerations in participants' locomotor profile.

Therefore, in the present study, we proposed to supplement male and female ballet dancers' habitual training with brief bouts of diverse, high-intensity exercise, modified from the protocol in sedentary females to increase the duration of vertical and diverse movement phases with flight and impact. Whereas third year vocational students were allocated to receive supplemental exercise, second year students trained as usual, and to investigate the effects of 12 weeks ballet training, with and without superimposition of a targeted, low-volume exercise stimulus on bone, we sampled background biomarkers of bone metabolism, gonadal hormones and serum 25(OH)D (vitamin D), as well as measuring calcaneal stiffness. According to previous evidence in sedentary females, we hypothesised that locomotor profile would exhibit a training effect of diverse exercise, specifically, an increase in duration of vertical accelerations,

and that the intermittent protocol would elicit high-training intensity, characterised by heart rate during exercise and elevation in blood lactate concentration. We therefore quantified accelerations, heart rate, and blood lactate responses during a bout of intermittent exercise at baseline, and after 12 weeks' training, to address the question: is there is an effect of brief, diverse exercise on bone in vocational ballet students, and is this associated with adaptations in vertical acceleration profile.

8.2 Methods

8.2.1 Study design

This study was a non-randomised training intervention, conducted in a population of male and female ballet dancers in their final two years of pre-professional training, that adopted convenience sampling. A power calculation was not performed as it was decided, a priori, to accept onto the study all dancers meeting inclusion criteria, who were eligible to participate and gave informed consent. Furthermore, as representative data for interventions with supplemental exercise in this population are lacking, and the diverse exercise regimen was novel, mean and standard deviations for key outcome measures (accelerations during exercise, bone biomarkers and QUS) from athlete populations were not available to derive effect size and calculate power.

The senior year group of dancers consented onto the study received supplemental exercise alongside usual dance training (TAU), whereas consented dancers in the year below received TAU. After consultation with staff at the school, and examination of year group timetables, it was established that volume of TAU was the same in both year groups (35 h per week) (see Study Flow Diagram, Figure 26).

8.2.2 Recruitment

Female and male dance students at The Central School of Ballet (CSB), London, were invited to take part in the study, via a letter circulated to students and, if under 18 years of age, to parents or guardians, which explained investigative aims and procedures. Participants wishing to take part completed a Participant Screening Questionnaire (PSQ) to assess eligibility, and where participants were under 18 y, this was also signed by a parent or guardian. After completing the PSQ, participants received the participant information sheet,

which explained all experimental procedures and the exercise training intervention. Participants were informed that all data they provided would be anonymised and securely stored, in accordance with ethics procedures, and told that they could withdraw from the study at any time without giving reasons for this decision. Informed, written consent, countersigned by parent or guardian if under 18 y, was obtained from participants admitted to the study, which was approved by the local ethics committee (see Appendix A2.4).

Dancers meeting the following inclusion criteria were accepted onto the study:

- aged 17-25 y
- full-time student in dance training at CSB
- generally healthy
- no medical condition or injury preventing participation in High Intensity Interval Training (HIIT) with a diverse-movement focus

Student dancers were excluded from participation if answering yes to the following:

- currently injured
- stress fracture or achilles tendon rupture in the previous 6 months

Dancers who indicated they did not want to take part or whose parents did not provide consent (under 18 y) were not accepted onto the study.

8.2.3 Assignment

Students in the final (third) year at CSB consented on to the study and meeting inclusion criteria were assigned to two sessions per week of DM-HIIT, as a supplement to full-time dance training. Students in the second year consented on to the study received full-time dance training as usual (TAU) and did not attend DM-HIIT. Participants in both interventional arms were given a number on acceptance onto the study to ensure data were annonymised.

8.2.4 Schedule for data collection

Data collection was performed in all participants at baseline and after 12 weeks (Methods 8.2.5 - 8.2.12, inclusive). Blood collection was undertaken at

the start of the week to allow a minimum of 48 hours after DM-HIIT and usual training.

To examine detraining effects on biomarkers of bone turnover (P1NP, CTX-1) and measurement of 25(OH)D (25-hydroxy vitamin D) in DM-HIIT females and males, a further round of blood collection (Methods 8.2.9) was undertaken three weeks after cessation of DM-HIIT, on return from the Christmas holidays.

8.2.5 Anthropometric data collection

On arrival at the school, before the start of daily classes, body weight (kg) and height (m) were measured, and BMI calculated (see General Methods 3.2.1 and 3.2.2).

Waist and hip circumference were measured (see General Methods, 3.2.4) and waist to hip ratio calculated (W:H, AU). All measurements were performed by the Lead Physiotherapist at the school, for consistency in anthropometric data collection, and in accordance with CSB safe-guarding procedures.

8.2.6 Heart rate and blood pressure at rest

Heart rate and blood pressure at rest were measured after participants had sat quietly for ten minutes (see General Methods 3.2.5).

8.2.7 Assessment of medical history, dietary and activity status

Participants completed a questionnaire (see Appendix A1.1) to provide information about medical history, menstruation status, consumption of alcohol and tobacco, use of contraception and activity status. Calcium consumption was investigated using a questionnaire (see Appendix A1.2) validated for evaluation of dietary calcium (Sebring et al., 2007). Readiness to undertake physical activity was assessed using a form (PAR-Q, V 2002) validated for this purpose (Thomas, Reading, & Shephard, 1992) (See Appendix A1.1).

8.2.8 Quantitative ultrasound of the calcaneus

After undertaking manufacturer's QA procedure quantitative ultrasound scan (GE Lunar Achilles Insight, Bedford, UK) was performed on the left and right calcaneus (see General Methods 3.2.8).

8.2.9 Venipuncture

Venipuncture undertaken to obtain serum samples for analysis of biomarkers of bone turnover (P1NP, CTX-1), gonadal steroid hormones (females: 17 β-estradiol [E2], luteinising hormone [LH] follicle stimulating hormone [FSH]; males sex hormone binding globulin [SHBG] and total testosterone [T]) and serum 25(OH)D.

Participants were given scheduled appointments, in order to standardise time of sampling for entry and exit of the study, and were instructed to arrive in a fasted state (water only after midnight) for blood collection, before the start of daily dance classes. After inspection of the antecubital region, venipuncture was performed (see General Methods 3.3.1); blood samples were drawn into yellow top serum vacutainers and handled as previously described (General Methods 3.3.1, paragraph 4). After being allowed to stand for 60 minutes at room temperature, vacutainers were packed on ice and transported to a local laboratory, where they were centrifuged at 1300 RCF for 10 minutes at 4°C. Serum supernatants were pipetted into individually labelled eppendorfs (1 mL per sample), which were then packed in ice and transported to a freezer to be stored at -80˚C for subsequent analysis (see General Methods 3.3.2).

8.2.10 Schedule for exercise testing

At baseline, dancers who received DM-HIIT undertook a quantified bout of diverse exercise previously examined in sedentary females (Chapter 6, 6.2.8) and the same protocol was again applied as a ‘test-retest’ exposure after 12 weeks’ training with ramped DM-HIIT as described in section 8.2.13.

Participants were familiarised with the DM-HIIT test bout by DVD and a streaming link prior to testing, which gave access to a short film that provided an audio-visual demonstration.

Before exercise, participants allocated to both supplemental DM-HIIT and TAU were fitted with a heart rate monitor (Polar T31, Polar, Finland) and a movement sensor unit (OptimEyeS5, Catapult Sports, Australia) worn in a close-fitting vest, supplied by the manufacturer (see General Methods 3.5.1). Participants were instructed to stand still for 10 s after the unit was switched on and then performed a bout of submaximal exercise (see General Methods 3.7

paragraph 2). A capillary blood sample for lactate measurement (see General Methods 3.4.2) was obtained immediately after submaximal exercise.

In dancers allocated to TAU, units were switched off immediately after submaximal exercise and training vests removed, whereas dancers allocated to supplemental DM-HIIT, undertook a bout of diverse-movement HIIT (DM-HIIT); indicative duration of activity phases is described in Chapter 5, Table 14.

Each DM-HIIT participant exercised during the test within a 3 m x 3 m space marked out as a grid, using flat yellow tape adhered to the floor to mark the edges. The grid was aligned to allow unimpaired visual and audio access to the test demonstration and commentary, projected on a screen in front of the exercise area. Immediately after completing DM-HIIT the motion sensor unit was switched off.

8.2.11 Lactate sampling

A capillary lactate sample was obtained (see General Methods 3.4.2) immediately after submaximal exercise and two further samples taken immediately, and two minutes after, DM-HIIT. Participants remained standing whilst samples were obtained.

8.2.12 Rating of exercise effort

Rating of exercise effort was undertaken using a visual analogue scale (see General Methods 3.6.2) between obtaining the second and third capillary lactate sample. Participants remained standing during rating of effort.

8.2.13 Supplemental DM-HIIT intervention

Weeks 1 – 4 Ramp 1 of DM-HIIT consisted of 7 x 60 s bouts of dynamic actions, prescribed according to the 10-20-30 principle of progressive effort (Gunnarsson & Bangsbo, 2010) and 250 s of balance and mobility (active recovery, [AR]), scheduled in a mean work to AR (W:AR) ratio of 1:0.6, including brief (4 s) transitions between phases of choreography to allow for titles of sections. Duration for DM-HIIT Ramp 1 was 720 s.

Weeks 5 - 8 Ramp 2 comprised 435 s dynamic DM-HIIT, scheduled in 5 x 60 s bouts of progressive intensity actions, as described for Ramp 1 above, 1 x 50 s dynamic hopping and flighted bounding, and 1 x 85 s dynamic lunging, incorporating uni- and bi-lateral jumping. AR comprising 220 s spinal mobility,

balance and quadrupedal body weight loading was prescribed as a mean W:AR of 1:0.5. Duration for Ramp 2, including transitions between phases of choreography, was 720 s.

Weeks 9 - 12 Ramp 3 comprised 6 bouts of 60 s actions, ramped by prescribing faster speed of execution, briefer contact and longer flight phases for dynamic actions. Intense actions included 140 s of bounding, bilateral jumping and flighted hopping (prescribed as 40, 50 and 50 s bouts, respectively), giving a total Ramp 3 of 500 s dynamic actions and 140 s of active recovery, balance and mobility, prescribed in a mean W:AR of 1:0.3. Total duration for Ramp 3 with transitions was 690 s.

Training took place in groups (maximum of seven); each participant exercised within a 3 m x 1.5 m floor area, marked out with flat tape. The same experimenter and CSB staff member (Lead Physiotherapist) attended all sessions and encouragement and coaching of exercise actions was standardised. Briefly, participants were encouraged to brake movements rapidly and maintain a stiff (versus soft) shape in the torso and lower limbs during landing. In addition, brief ground contact, high cadence and maximal height of jumping and hopping and ‘as fast as possible’ COD were coached during dynamic actions and lunging.

All supplemental DM-HIIT sessions took place in the same studio, between 10 and 11.30 a.m., and were 48 h apart. Participants were instructed to wear the same shoes during DM-HIIT, and a register of training attendance was kept.

Films of the ramps and baseline DM-HIIT protocol can be viewed via the link provided in the Appendix(A3.3).

8.2.14 Rating of effect of diverse HIIT on dance fitness

Dancers were asked to rate their perception of DM-HIIT training according to ‘improvement of dance fitness’, using a non-validated VAS (Appendix A1.3). The scale consisted of a horizontal line extending from left (0 = 0% change) to right (100 = 100% change) and participants were instructed to place a mark along the line at a point representing their perception of the effect of training on dance fitness. Rating was undertaken at exit to the study, after three weeks detraining, on the return to the school.

8.2.15 Acceleration profile

Participant data acquired during submaximal and DM-HIIT exercise at 0-, 12- w were downloaded (Catapult Sprint 5.1.7, Catapult Sports, Australia). For participants assigned to supplemental DM-HIIT, data were split into two files, corresponding to submaximal and DM-HIIT bouts, for participants receiving TAU, a single file for submaximal exercise was obtained during download. Data were exported from the manufacturer's download environment for further data analysis, as described in General Methods 3.5.3. Acceleration bands for durations of accelerations during submaximal exercise and DM-HIIT were characterised as summarised in Chapter 6, Table 15.

8.2.16 Statistical approach

Data were analysed using a statistical software package (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). For outcome measures provided by participants in both intervention groups (HR and BP at rest, anthropometrics, QUS, biomarkers of background bone turnover, gonadal steroid hormones and 25(OH)D status) data from females and males, collected at baseline and after 12 weeks, were separated and compared using two-way mixed-model analysis of variance (ANOVA), with 'intervention group' as the between subject factor and 'time' as the within-subjects factor.

The effect of de-training on serum biomarkers of bone turnover, gonadal steroid hormones and 25(OH)D was examined using one-way repeated measures ANOVAs within males, and separately, within females allocated to supplemental DM-HIIT, who provided detraining blood samples (detraining data not collected in TAU).

Analysis of acceleration data was planned a priori to enable comparison of:

1. acceleration profile in male and female dancers exposed to a bout of DM-HIIT in the untrained state
2. the effects of 12 weeks DM-HIIT on accelerations during submaximal exercise within female dancers, and separately, within male dancers

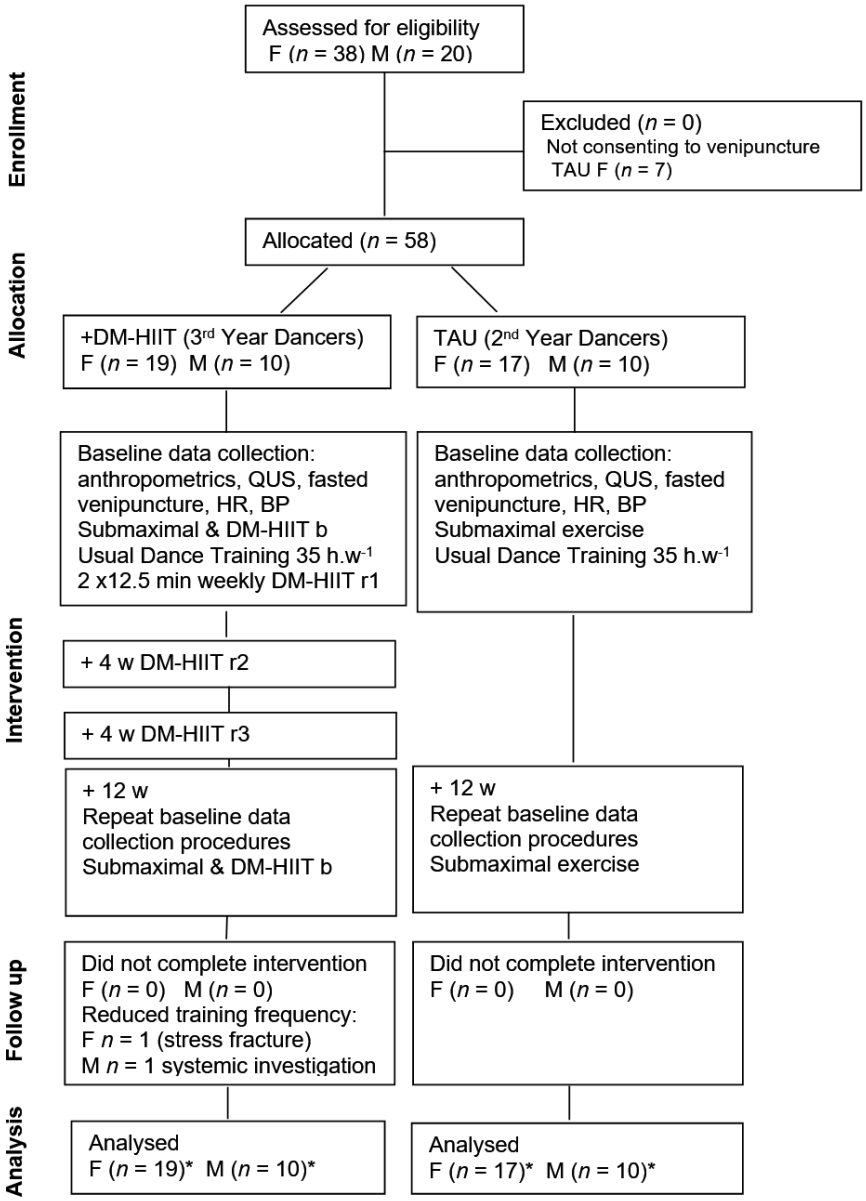
3. accelerations in the untrained and trained state during a single bout of DM-HIIT within females, and separately, within males, allocated to 12 weeks supplemental DM-HIIT

To address 1. (above), for acceleration data from DM-HIIT testing at 0 weeks, separate three-way mixed-model analysis of variance (ANOVA) were used, with ‘sex’ as the between group factor, and ‘direction’ and ‘acceleration band’ (linear data) or ‘plane of motion’ and ‘radian band’ (angular data) as repeated-measures factors. Two-way ANOVAs were used post hoc where a significant three-way interaction was found, and to characterise second order interactions. Bonferroni corrections were applied.

To address 2. (above), three-way ANOVAs were used for submaximal data in females, and separately in males, with ‘group’ as the between-subjects factor. Separate three-way ANOVAs were performed for each orthogonal (or plane of motion), and each acceleration band (or radian band), therefore within-subject factors were ‘time’ and ‘band’ for the former comparison, and ‘time’ and ‘direction’ for the latter.

To address 3. (above), three-way repeated measures ANOVAs were performed on DM-HIIT acceleration data at 0 weeks and 12 weeks, within males, and separately, within females. The three within-subjects factors were ‘time’, ‘direction’ and ‘acceleration band’, and this approach was adopted for both linear, and angular, acceleration data. For T-tests to compare accelerations in males, and separately in females, within orthogonal, post hoc correction was applied and a lower p value accepted ($p < 0.025$).

For all statistical analyses, p was set at 0.05 and T-tests were two-tailed.



Note. * Where participant data were unavailable (e.g. not consenting to venipuncture) *n* for participants analysed is indicated in results.
DM-HIIT: Diverse-Movement HIIT; *b*: basal protocol implemented 0- and 12 weeks. R1,2,3 Ramp of DM-HIIT.

Figure 26 Flow Diagram: DM-HIIT Supplementation in Ballet Dancers

8.3 Results

8.3.1 Participant characteristics

Data for baseline characteristics were separated by sex to compare between intervention groups (training as usual [TAU] versus DM-HIIT).

All data were normally distributed, and no outliers were detected in the data sets (Table 29). In DM-HIIT females, weight was significantly higher than in TAU females (53.1 ±5.6 kg versus 49.5 ±4.5 kg; $p = 0.041$), no other differences between female dancers were observed in baseline characteristics.

In males, heart rate at rest was significantly lower in TAU compared with DM-HIIT (78 ±7 bpm versus 89 ±7 bpm; $p = 0.002$), no other differences in baseline characteristics were observed (Table 29).

Reported menstrual status and oral contraceptive (OC) use In DM-HIIT females, 68% ($n = 13$) reported regular periods, 26% ($n = 5$) irregular periods and one female had not achieved menarche. In TAU females, periods were reported as regular in 50% ($n = 8$), irregular in 31% ($n = 5$), absent in 13% ($n = 2$) and one female had not achieved menarche; one TAU participant was non-respondent. Current OC use was reported by 26% ($n = 5$) of DM-HIIT and 19% ($n = 3$) of TAU females; inspection of questionnaires showed that all OC users reported ‘regular periods’ in questions relating to menstrual cycle.

Weekly activity Respondents in all interventional groups declared participation in 30-35 hours per week of physical activity, specified as ‘full-time ballet and dance training’. After discussion with staff, this was explained to consist of 17 h formal ballet training (comprising daily classes and specific ballet preparation, such as partner and pointe work), classes in other dance disciplines (3 h contemporary and 1 h jazz per week), supplementary body conditioning for dance (1 - 2 h Pilates based exercise per week), with the remaining hours spent in choreographic practice and rehearsal for dance-related study projects and dance education.

Four TAU females declared supplemental physical activity, consisting of gym, strength training and Pilates for 30 - 60 min per day ($n = 3$), and one

participant, who had not achieved menarche, exercised for 60 min per day, in addition to full-time dance training.

Table 29 Comparison Between Female Dancers and Between Male Dancers At Baseline (Mean ±SD)

	Female			Male		
Variable	DM-HIIT (*n* = 19)	TAU (*n* = 17)	*p* value	DM-HIIT (*n*=10)	TAU (*n*=10)	*p* value
Age y	18.6 ±1.2	17.7 ±0.8	0.006	18.4 ±0.8	17.5 ±0.8	0.028
Height m	1.66 ±0.07	1.64 ±0.05	0.186	1.77 ±0.08	1.73 ±0.05	0.120
Mass kg	53.1 ±5.6	49.5 ±4.5	0.041	66.8 ±7.4	62.2 ±5.5	0.131
BMI $kg.m^2$	19.1 ±1.7	18.5 ±1.4	0.307	21.2 ±1.4	20.9 ±1.7	0.683
Waist to hip AU	0.79 ±0.04	0.78 ±0.03	0.325	0.84 ±0.02	0.86 ±0.05	0.311
Menarche	13.8 ±1.6	13.9 ±1.4	0.769	- -	- -	- -
HR bpm	82 ±10	76 ±14	0.152	89 ±7	78 ±7	0.002**
Sys BP mmHg	105 ±12	102 ±11	0.424	121 ±9	121 ±5	0.977
Dia BP mmHg	65 ±10	63 ±8	0.389	71 ±8	68 ±9	0.480
Ca $mg.d^{-1}$	1556 ±798	1426 ±707	0.659	2148 ±1248	1661 ±706	0.349

DM-HIIT: Supplemental Diverse-Movement HIIT; TAU: Training As Usual; AU: Arbitrary Units; HR: Heart rate; BP: Blood Pressure; SYS: Systolic; DIA: Diastolic; Ca: Daily Calcium Intake

8.3.2 Adverse events, attrition, attendance

There were no adverse events associated with DM-HIIT training and no participants were lost to follow-up due to injuries or incidents related to DM-HIIT. Four female participants experienced injuries associated with dance training (three soft tissue, one bone stress response) and compliance with rehabilitation advice affected regular DM-HIIT attendance (mean time loss 13 ±2 sessions; 51 ±7% of total DM-HIIT sessions provided).

Females Mean attendance was 21 ±5 sessions (82 ±20%), which increased to 24 ±3 sessions (91 ±10%) on exclusion of the four participants with significant (n = 1) dance injury-related DM-HIIT time loss, as described. Four female participants (21% of DM-HIIT females) attended 100% of 26 sessions.

Males No injuries were reported which prevented DM-HIIT. Mean attendance was 20 ±5 sessions (75 ±20%), which included one male participant investigated for a health issue unrelated to the intervention. This reduced his availability for DM-HIIT participation (14 sessions, 54% of total DM-HIIT, not attended). One male participant attended infrequently (15 sessions, 58% of total DM-HIIT, not attended) due to timetabling affecting compliance with the protocol. No male participant recorded 100% attendance.

Data concerning injury, and associated time loss from regular dance training, was not obtained for TAU, who were consented for pre- and post-intervention data gathering only.

8.3.3 Physiological demands of DM-HIIT

Exercise heart rate

In the DM-HIIT group, significant main effects of the intervention were observed for all training heart rate (HR) parameters ($p < 0.05$), which were reduced during DM-HIIT after 12 weeks ($p < 0.05$) (Table 30). Peak exercise HR was 6 ±2 bpm lower ($p = 0.014$), minimum HR was 14 ±4 bpm lower ($p = 0.003$), and the largest effect was for mean HR during exercise, which reduced by 11 ±2 bpm ($p < 0.001$).

No significant effect of gender was found for any HR parameter, however there was a slight trend towards a greater reduction in males than females for mean exercise HR ($p = 0.070$, $\eta^2 = 0.142$; time*sex interaction effect), which

reduced from 179 ±11 to 164 ±11 bpm (p = 0.024), compared with 166 ±18 to 160 ±14 bpm in females (p = 0.028) (Table 30).

Training intensity, characterised as mean exercise HR as a percentage of peak HR recorded during exercise, was also significantly reduced after 12 weeks (p = 0.003, η^2 = 0.332, main effect of time) (Table 30), in females intensity reduced below 85 – 95% threshold proposed for HIIT (Ito, 2019), decreasing from 86 ±5% at baseline to 84 ±6% (p = 0.125) and remained within HIIT thresholds for males (85 ±4% versus 89 ±2%, p = 0.061; 12 w versus 0 w, respectively).

Table 30 Heart Rate During Diverse-Movement HIIT Pre- and Post- 12 weeks (Mean ±SD)

	Female		Male		Main Effect Time		Interaction Effect		Main Effect Group	
Variable	0 w	12 w	0 w	12 w	*P*	η^2	*p*	η^2	*p*	η^2
HR peak bpm	193 ±12	191 ±9	202 ±12	192 ±10	0.014	0.243	0.139	0.097	0.255	0.058
HR min bpm	106 ±21	93 ±16	113 ±14	98 ±13	0.003	0.346	0.752	0.005	0.291	0.050
HR mean bpm	166 ±18	160 ±14	179 ±11	164 ±11	<0.001	0.493	0.070	0.142	0.172	0.083
HR_{MEAN}/HR_{PEAK}%	86 ±5	84 ±6	89 ±2	85 ±4	0.003	0.332	0.373	0.036	0.316	0.046

HR: Heart Rate; min: minimum; HR_{PEAK}: Peak HR recorded during exercise.
Note. Females *n* = 18; Males *n* = 10; provided pre- and post- acceleration data.

8.3.4 Locomotor profile

DM-HIIT at baseline

Linear profile On first exposure to DM-HIIT at baseline, no effect of sex was observed on acceleration profile, as described by the three-way interaction between orthogonals and gbands on duration of accelerations (p = 0.309, η^2 = 0.043, sex*orthogonal*gband). During DM-HIIT, dancers of both sexes performed more accelerations at the lowest amplitude (1 to 1.5 g) than in gbands above this threshold (p < 0.001, η^2 = 0.954, main effect of gband). Across all three orthogonals, more accelerations were performed at each gband, compared with the gband above (p < 0.001, pairwise comparison between gbands). However, whilst no direction-specific effect of sex was found for duration of accelerations (p =0.254, η^2 = 0.051, orthogonal*sex), an effect of sex was observed on duration of linear accelerations during exercise (p = 0.003, η^2 = 0.297, main effect of sex) across the five gbands characterised (p = 0.001, η^2 = 0.328, sex*gband). Below 3 g forwards and sideways, duration of accelerations during DM-HIIT was greater in males than in females within each gband (p < 0.05, males versus females, duration of accelerations, for 'forwards' and for 'sideways', below 3 g) however, time spent in vertical acceleration did not differ significantly between males and females at any amplitude (p > 0.05, males versus females, gbands within 'up').

In both sexes, DM-HIIT was characterised by a non-uniform, i.e. diverse, distribution of accelerations across orthogonals (p < 0.001, η^2 = 0.881, main effect of orthogonal) and was non-monotonic i.e., varied according by gband (p < 0.001, η^2 = 0.959, main effect of gband). Pairwise comparison showed duration of vertical accelerations was 10.6 ±0.7 s longer than for lateral accelerations (p < 0.001), and 7.9 ±0.6 s longer than for forwards accelerations (p < 0.001). Duration of lateral accelerations was 2.7 ±0.3 s less than forwards (p < 0.001) and 10.6 ±0.7 s less than vertical (p < 0.001) indicating that side to side actions incurred the least speed changes.

Angular profile As for linear acceleration data, there was no effect of sex on angular acceleration profile, as described by the interaction between rotational plane (Rplane) and angular acceleration band (Rband) (p = 0.169, η^2 = 0.065, Rplane*Rband*sex). However, unlike results for linear accelerations,

male and female dancers exhibited different trunk angular motion strategies during exercise (p = 0.009, η^2 = 0.0.176, Rplane*sex), which was amplitude specific (p = 0.028, η^2 = 0.152, Rband*gender). In response to exercise choreography, males performed significantly more sagittal (forwards-backwards) than frontal plane (side-bending) trunk accelerations and decelerations between 90 and 150 degrees (p < 0.05, pairwise comparison between angular planes in males), whereas in females, trunk side-bending and forwards and backwards trunk motion were utilised equally during larger amplitude angular trunk accelerations (p = 1.000, pairwise comparison between 'yaw' and 'pitch', 90 to 150 degrees Rbands in females). At the highest amplitude (>150 degrees), duration of angular accelerations in females was 6.8 ±2.1 s for sagittal, compared with 5.2 ±1.3 s frontal plane angular accelerations (p = 0.806, pairwise comparison), whereas in males, comparable durations were 7.9 ±1.7 s sagittal and 3.7 ±1.3 s frontal (p = 0.070, pairwise comparison).

This suggests that males and females tended to adopt different locomotor strategies during the most dynamic phases of DM-HIIT, with females exhibiting as much trunk side-bending as forwards and backwards (i.e. sagittal) inclination during acceleration-deceleration, compared with a predominance of sagittal trunk motion at higher amplitudes in males. Angular accelerations at the lowest amplitude predominated during DM-HIIT (p < 0.001, η^2 = 0.993, main effect of Rband), with lower durations at each R band, compared with the band below (p < 0.001, pairwise comparison between Rbands), in both males and females (p = 0.255, η^2 = 0.052, main effect of sex).

Effects of 12 weeks DM-HIIT on basal acceleration profile

Females There was a highly significant effect of training state on the duration of linear accelerations across orthogonals and gbands (p < 0.001, η^2 = 0.620, orthogonal*gband*training state), and a direction-specific (p < 0.001, η^2 = 0.676, orthogonal*training state) and amplitude-specific (p < 0.001, η^2 = 0.713, gband*training state) effect on duration of accelerations. Whilst lateral accelerations were not different in the trained state (p = 0.211, η^2 = 0.090, training state*gband within 'sideways'), a significant interaction was observed for duration of forwards (p = 0.046, η^2 = 0.214, training state*gband within 'forwards') and vertical (p < 0.001, η^2 = 0.764, training state*gband within 'up') accelerations. After 12 weeks females performed 4.3 ±1.9 s more forwards (p =

0.034, pairwise comparison) and 21.6 ±3.0 s more vertical ($p < 0.001$, pairwise comparison) accelerations between 1 and 1.5 g during DM-HIIT. Post-hoc comparison showed duration of vertical accelerations increased at every gband below 3 g ($p < 0.025$), and above 3 g increased by 1.0 ±0.2 s after the intervention, compared with baseline ($p = 0.001$).

There was no effect of training state on the three-way interaction describing angular accelerations during DM-HIIT ($p = 0.114$, $\eta^2 = 0.111$, rotary plane*angular band*training state). However, there was an amplitude-specific effect ($p < 0.001$, $\eta^2 = 0.715$, angular band*training state), whereby duration increased at each Rband below 90 degrees ($p < 0.001$, pairwise comparison between training states), and between 90 and 150 degrees ($p < 0.05$) but did not increase above 150 degrees in any angular plane ($p = 0.450$). As observed at baseline, trunk motion during exercise was predominantly characterised by low amplitude (30 – 60 degree) angular accelerations ($p < 0.001$, $\eta^2 = 0.997$, main effect of angular band) and a pattern of stepwise, significant decreases in duration at each angular band, compared with the band below ($p < 0.001$, pairwise comparison between Rbands).

Males A significant effect on linear acceleration profile was also observed in males after 12 weeks training ($p = 0.005$, $\eta^2 = 0.532$, training state*orthogonal*gband) which, as in females, was direction-specific ($p = 0.005$, $\eta^2 = 0.532$, training state*orthogonal). However unlike in females, there was not an amplitude-specific effect after 12 weeks training ($p = 0.081$, $\eta^2 = 0.329$, training state*gband). Duration of vertical accelerations during DM-HIIT increased ($p = 0.005$, $\eta^2 = 0.532$, training state*orthogonal) by 5.1 ±1.7 s ($p = 0.016$), compared with an increase of 1.5 ±0.9 s forwards ($p = 0.144$) and 0.6 ±0.3 s sideways ($p = 0.098$). Below 3 g, whilst vertical accelerations increased at each gband ($p < 0.025$), the largest increase (16.1 ±17.1 s) was observed between 1 - 1.5 g and above 3 g, unlike in females, did not change after 12 weeks training.

Unlike in females, there was a trend in males for an effect of training state on angular acceleration profile, as described by the interaction between angular acceleration variables ($p = 0.063$, $\eta 2 = 0.279$, Rplane *Rband*training state). However, as in females, whilst no directional effect on angular profile was observed ($p = 0.412$, $\eta^2 = 0.100$, Rplane* training state), 12 weeks training

did affect amplitude characteristics ($p < 0.001$, $\eta^2 = 0.729$, Rband*training state), which increased at each Rband below 150 degrees ($p < 0.01$) but not above that ($p = 0.065$). In males, as in females, durations differed across thresholds categorised ($p < 0.001$, $\eta^2 = 0.993$, main effect of Rband). Specifically, there was a stepwise reduction in duration of accelerations as Rband increased in amplitude ($p < 0.001$, pairwise between Rbands), a locomotor feature of DM-HIIT that was not affected by training condition.

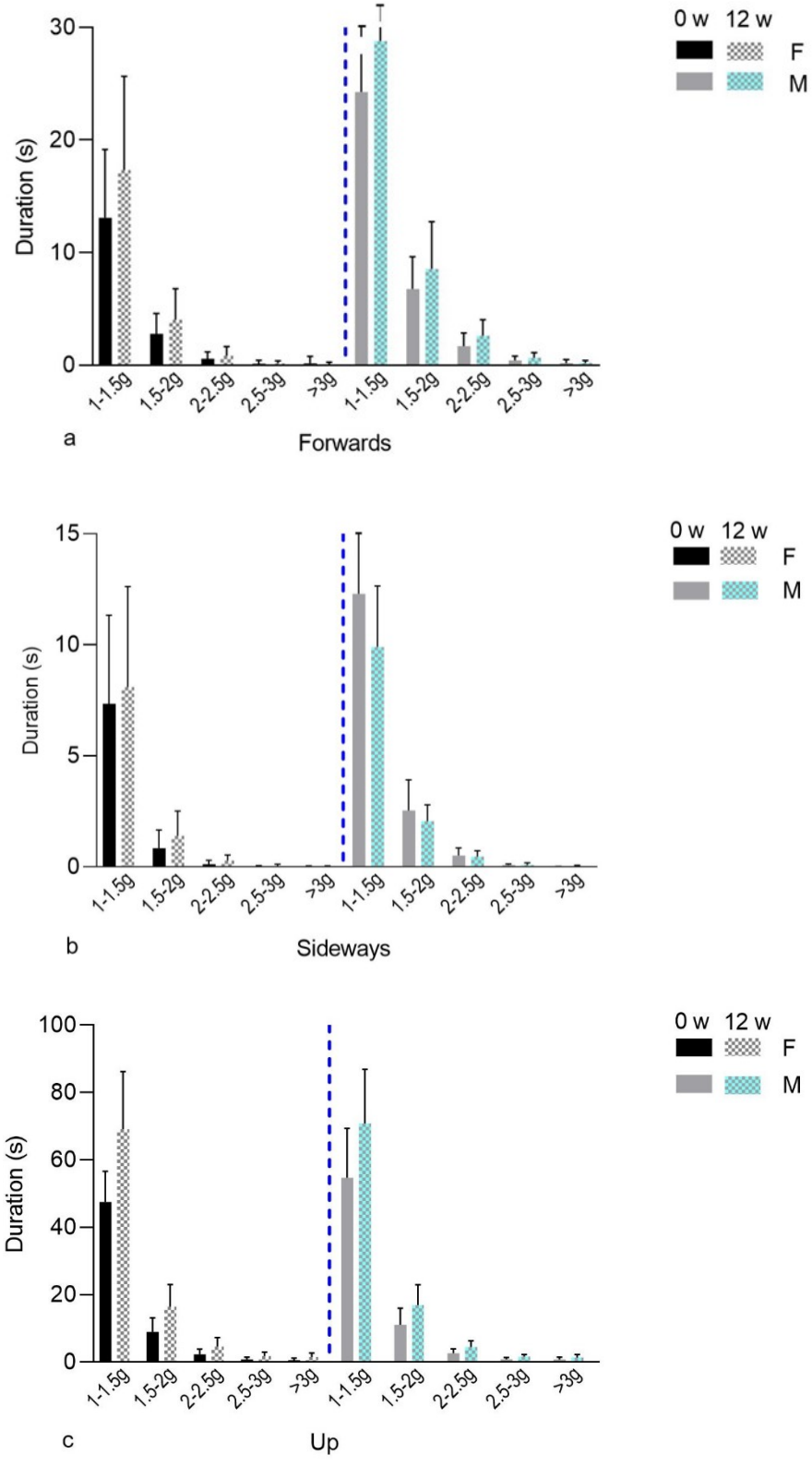
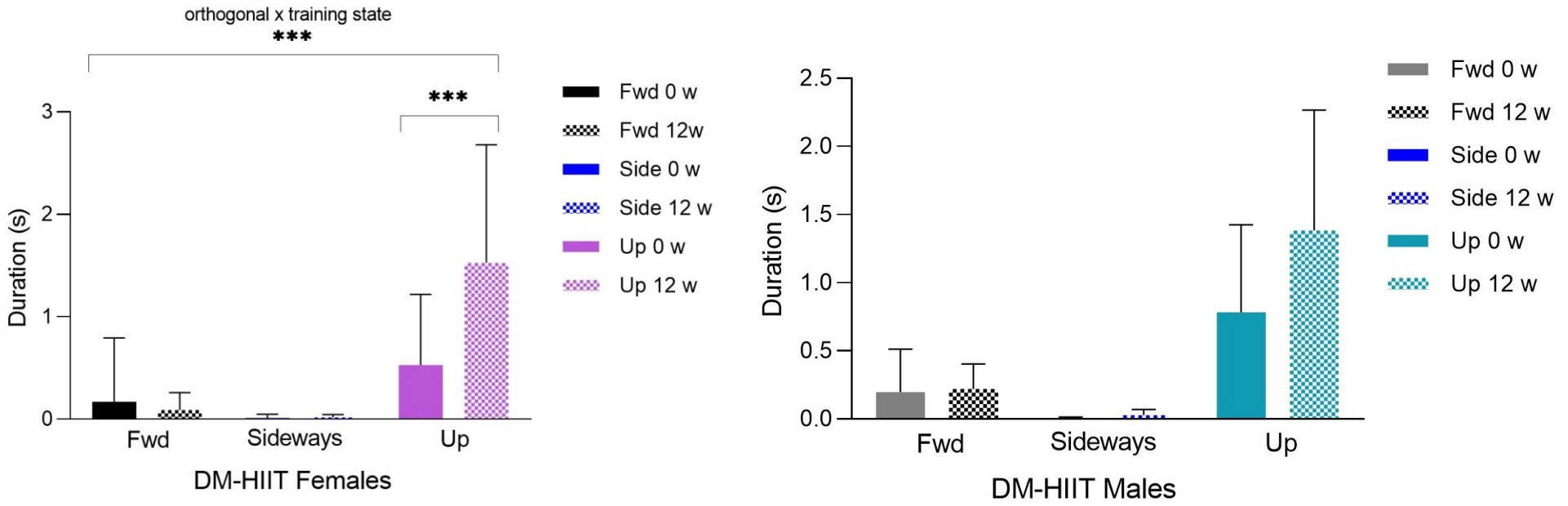


Figure 27 Linear Accelerations at 0- And 12-Weeks (Mean +SD)



DM-HIIT: Diverse-Movement HIIT; Fwd: Forwards; Side: Sideways; Up: Vertical.
*** ($p < 0.001$)

Figure 28 Duration of Accelerations Above 3 g in Females and Males 0- Versus 12-Weeks (Mean ±SD)

8.3.5 Lactate concentration

In males and females capillary blood lactate concentration immediately after, and two minutes after, DM-HIIT did not change significantly after 12 weeks training, compared with baseline exposure in the untrained state (Table 31).

8.3.6 Perception of effort and estimation of training load

In both males and females, training state significantly affected perception of exercise demand imposed by DM-HIIT. After 12 weeks, rating of exercise challenge reduced by 10 ±5 % (p = 0.043, η^2 = 0.154, main effect of training state $VAS_{CHALLENGE}$), and perception of respiratory demand reduced by 14 ±4 % (p = 0.001, η^2 = 0.342, main effect of training state $VAS_{BREATHING}$), compared with baseline, however perceptual demand for work performed by legs did not decrease (Table 32). No difference between females and males was found for any category of rating of exercise demand (p > 0.05, main effect of gender) (Table 32).

There was no relationship, either at baseline or after 12 weeks training, between VAS score, or capillary blood lactate concentration immediately after DM-HIIT, and total duration of accelerations, either above 2 g, or above 3 g (p > 0.05).

Table 31 Capillary Blood Lactate Concentration After DM-HIIT (Mean ±SD)

Females BLa				Males BLa									
0 w		12 w		0 w		12 w		Time*Sex		Time		Sex	
0 min	+2 min	0 min	+2 min	0 min	+2 min	0 min	+2 min	*p*	η^2	*p*	η^2	*p*	η^2
8.1 ±4.5	5.5 ±3.6	6.5 ±2.5	5.3 ±2.3	8.8 ±2.8	7.2 ±3.7	8.3 ±2.8	6.2 ±2.4	0.910	0.001	0.265	0.052	0.224	0.061

Note. 0 min, +2 min: time of sampling after cessation of DM-HIIT exercise.
BLa: Capillary blood lactate concentration (mmol.L^{-1}).

Table 32 Perception of Exercise Demand (Mean ±SD)

	Females (*n* = 18)		Males (*n* = 9)		Interaction		Sex		Time	
$VAS_{CATEGORY}$	0 w	12 w	0 w	12 w	*p*	η^2	*p*	η^2	*p*	η^2
Challenge AU	58 ±15	42 ±17	55 ±22	51 ±13	0.219	0.060	0.584	0.012	0.043	0.154
Legs AU	46 ±17	45 ±17	53 ±19	49 ±18	0.768	0.004	0.208	0.063	0.703	0.006
Breathing AU	58 ±16	47 ±18	67 ±13	50 ±18	0.509	0.018	0.509	0.018	0.001	0.342

VAS: Visual Analogue Scale; AU: Arbitrary Units.

8.3.7 Calcaneal Quantitative Ultrasound

Baseline versus 12 weeks

Males In DM-HIIT supplemented males, left calcaneal stiffness tended to be significantly increased by 16 ±26% after 12 weeks, compared with a reduction of 2 ±5% in TAU (p = 0.055; η^2 = 0.223; time* group interaction effect) (Table 33) and left BUA increased by 26 ±68% in DM-HIIT males, compared with a reduction of 4 ±5% in TAU (p = 0.089; η^2 = 0.181; time*group interaction effect). Calcaneal speed of sound (SOS), a QUS parameter related to bone elasticity, increased on the left and right side in males after 12 weeks (p = 0.038; η^2 = 0.256; p = 0.041; η^2 = 0.249; main effect of time, left and right calcaneal SOS; respectively).

Data provided by one male dancer, who at baseline was an outlier for absolute difference between L and R Stiffness Index (Table 33), exhibited a large (> 2 SDs) increase in left SI after 12 weeks, and were re-checked for data entry errors. When none was identified, data were discussed with the school's Head of Medical Department, and it emerged that this individual had sustained a previous injury to the left ankle (resolved by time of entry to the study), entailing a period of off-loading. As non-outlier data was returned for the right calcaneus, the decision was taken to include left calcaneal data as representing a valid intra-individual result.

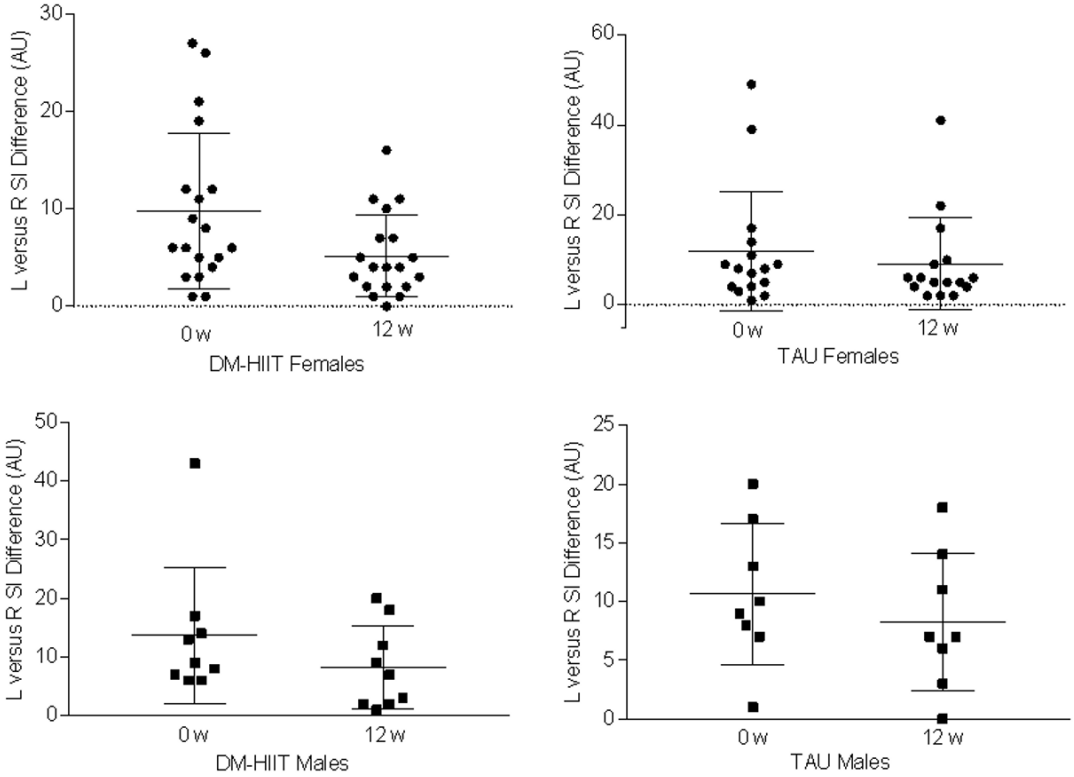
Females left BUA was lower in DM-HIIT than in TAU (p = 0.043; η^2 = 0.118; main effect of group), no other significant main or interaction effects for SI, BUA or SOS were observed (Table 33).

Left versus right SI After 12 weeks, absolute difference between left and right SI tended to be significantly reduced in males (p = 0.064; η^2 = 0.210; main effect of time) and reduced but not significantly in females (p = 0.131; η^2 = 0.068; main effect of time) (Table 33).

Table 33 Calcaneal Quantitative Ultrasound in Male and Female Dancers Pre- And Post- 12 weeks (Mean ±SD)

Female	DM-HIIT (*n* = 19)				TAU (*n* = 16)				Interaction		Group		Time	
QUS	0 w		12 w		0 w		12 w							
									p	η^2	*p*	η^2	*p*	η^2
L SI	103	± 6	105	± 3	111	± 5	109	± 6	.420	.020	.212	.047	.895	.010
R SI	102	± 7	105	± 5	106	± 5	108	± 2	.928	.000	.490	.015	.320	.030
L-R diff.	10		5		12	3	9	0	.702	.004	.151	.062	.131	.068
L BUA	112.47	± 6.40	117.46	± .83	122.47	± 7.14	124.91	± 3.42	.607	.008	.043	.118	.139	.065
R BUA	114.70	± 2.33	117.47	± 1.14	121.49	± 2.60	124.21	± 9.71	.992	.000	.097	.081	.316	.030
L SOS	1602.03	± 8.97	1597.31	± 1.24	1605.36	± 6.65	1592.79	± 3.63	.565	.010	.957	.000	.209	.047
R SOS	1588.49	± 9.50	1594.76	± 2.90	1591.46	± 5.12	1593.11	± 6.65	.491	.014	.959	.000	.241	.041
Male	DM-HIIT (*n* = 9)				TAU (*n* = 8)									
QUS	0 w		12 w		0 w		12 w							
L SI	113	± 5	124	± 4	103	± 0	100	± 6	.055	.223	.068	.205	.256	.085
R SI	117	± 0	123	± 9	101	± 8	103	± 9	.473	.035	.054	.225	.186	.114
L-R diff.	14		8		11		8		.445	.039	.667	.013	.064	.210
L BUA	123.79	± 8.01	135.33	± 6.25	120.56	± 7.94	115.42	± 2.89	.089	.181	.295	.073	.496	.031
R BUA	113.12	± 7.66	135.54	± 3.99	119.51	± 7.66	121.13	± 5.76	.898	.001	.074	.197	.520	.028
L SOS	1611.48	± 8.20	1621.59	± 3.24	1579.90	± 0.41	1582.24	± 1.02	.176	.118	.072	.199	.038	.256
R SOS	1603.10	± 9.85	1619.20	± 3.35	1576.43	± 2.37	1579.08	± 6.48	.130	.146	.087	.183	.041	.249

QUS: Quantitative Ultrasound Scan; SI: Stiffness Index (Arbitrary Units [AU]); diff: absolute difference between left and right SI; BUA: Broadband Ultrasound Attenuation ($dB.MHz.s^{-1}$); SOS: Speed of Sound ($m.s^{-1}$).



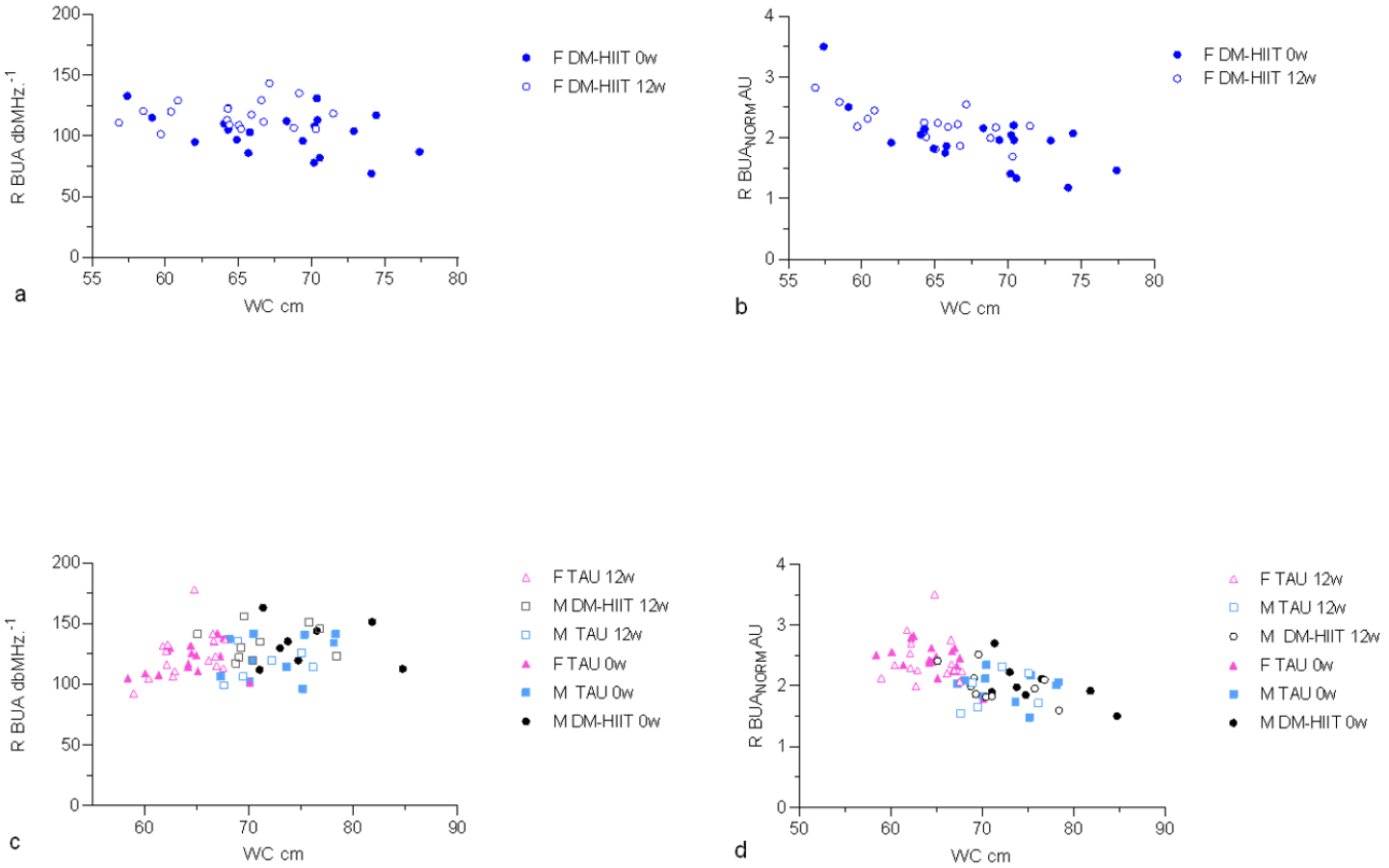
AU: Arbitrary Units; TAU; Training As Usual; DM-HIIT: Diverse-Movement HIIT

Figure 29 Absolute Difference Left vs Right Stiffness Index Pre- and Post-12 Weeks (Mean SD)

Calcaneal BUA and central adiposity

At baseline and after 12 weeks, no relationship between right calcaneal broadband ultrasound attenuation (R cBUA) and waist circumference (WC) was observed in any group ($p > 0.05$) (Figure 30 a, c), nor between WC, and R cBUA normalised to body mass (R $cBUA_{NORM}$) in either TAU females,TAU males or in DM-HIIT males (Figure 30 d).

In DM-HIIT females, WC and R $cBUA_{NORM}$ were negatively associated, both at baseline (r (19) = -0.67, p = 0.002), and after 12 weeks (r (19) = - 0.62, p = 0.004), indicating that an increase in the measurement of central adiposity, independent of body mass, was associated with a reduction in attenuation of the broadband ultrasound signal in calcaneal bone (Figure 30 b).



TAU: Training As Usual; DM-HIIT: Diverse-Movement HIIT; BUA_{NORM}: Broadband Ultrasound Attenuation normalised by body weight; WC: Waist Circumference.

Figure 30 Relationship Between Calcaneal BUA and Waist Circumference

8.3.8 Biomarkers of bone turnover

Baseline versus 12 weeks within males and within females

In males, CTX-1 reduced significantly after 12 weeks (p = 0.024; η^2 = 0.315; main effect of time) (Table 34), however there was no effect of DM-HIIT supplementation (p = 0.686; η^2 = 0.012; group*time interaction effect).

In DM-HIIT females, CTX-1 reduced after 12 weeks (p = 0.043, adjusted for multiple comparison) but did not change in TAU females (p = 0.853).

No significant interaction effect for P1NP, or main effect of time, was observed in either males or females however, serum P1NP was significantly higher in TAU males compared with DM-HIIT (p = 0.027; η^2 = 0.315; main effect of group) (Table 34).

Table 34 Comparison of Biomarkers of Bone Turnover 0- versus 12- Weeks (Mean ±SD)

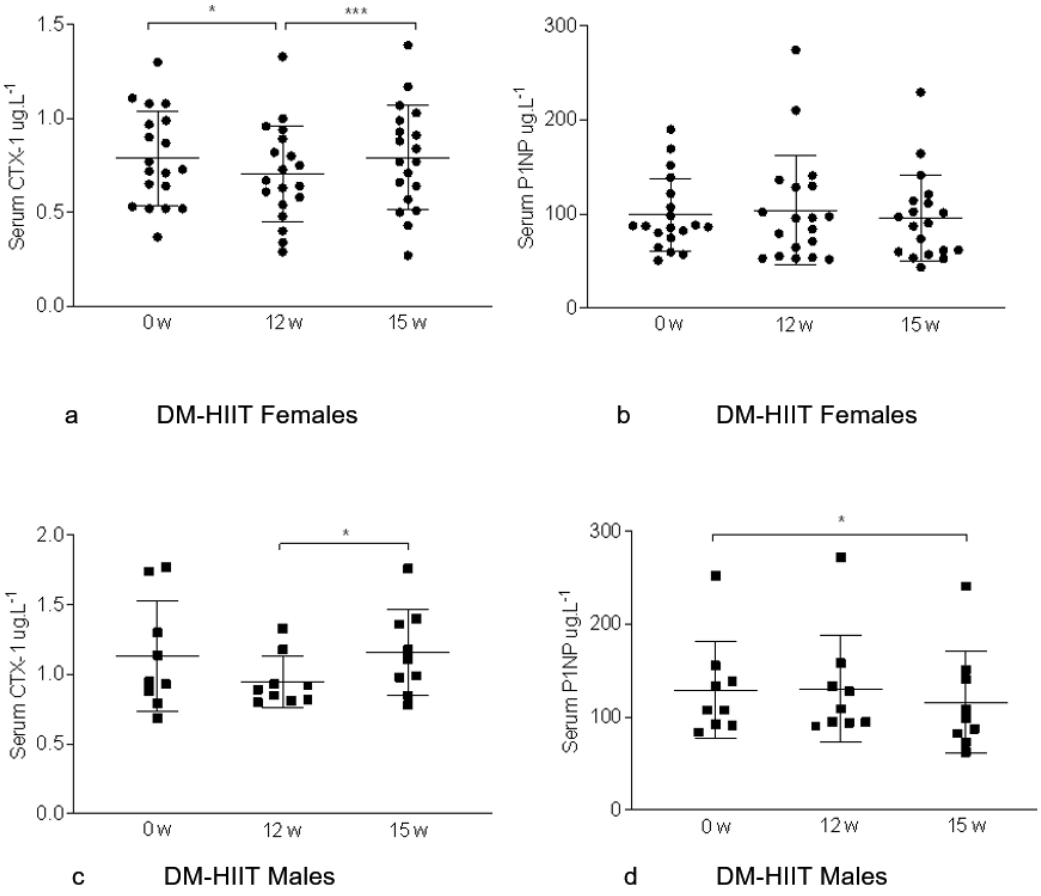
Sex	Biomarker	DM-HIIT (*n* = 19)		TAU (*n* = 11)		Time*Group		Time		Group	
		0 w	12 w	0 w	12 w	*p*	n^2	*p*	n^2	*p*	n^2
F	P1NP ug.L^{-1}	99 ±39	104 ±58	77 ±45	103 ±54	0.275	0.042	0.108	0.090	0.488	0.017
F	CTX-1 ug.L^{-1}	0.79 ±0.25	0.71 ±0.25	0.78 ±0.23	0.80 ±0.28	0.071	0.112	0.293	0.039	0.633	0.008
		DM-HIIT (*n* = 9)		TAU (*n* = 7)							
		0 w	12 w	0 w	12 w						
M	P1NP ug.L^{-1}	129 ±52	131 ±58	268 ±163*	235 ±116*	0.218	0.106	0.249	0.094	0.027	0.303
M	CTX-1 ug.L^{-1}	1.13 ±0.40	0.95 ±0.18†	1.41 ±0.37	1.27 ±0.45†	0.686	0.012	0.024	0.315	0.097	0.185

DM-HIIT: Diverse-Movement HIIT; TAU: Training As Usual; F: Female; M: Male; P1NP: N-Terminal Propeptide of Type 1 Procollagen; CTX-1: C-Terminal Telopeptide of Type I Collagen (β isoform).
* significant main effect of group ($p < 0.05$); † significant main effect of time ($p < 0.05$).

Effect of detraining on biomarkers of bone turnover

In DM-HIIT females, serum CTX-1 varied according to training state (p = 0.017; η^2 = 0.239, main effect of training state) and reduced after 12 weeks by 0.08 ±0.03 ug.L^{-1} compared with CTX-1 at baseline (p = 0.048) and increased by 0.08 ±0.03 ug.L^{-1} after three weeks detraining (p < 0.001) (Figure 31 a). No main effect of training was observed for serum P1NP (p = 0.136; η^2 = 0.209, main effect of training state), which increased by 5.09 ±11.9 ug.L^{-1} after 12 weeks DM-HIIT (p > 0.999) and reduced by 8.11 ±4.94 ug.L^{-1} after detraining (p = 0.355) (Figure 31 b).

In DM-HIIT males, as in DM-HIIT females, there was significant variation in CTX-1 (p = 0.028; η^2 = 0.361, main effect of training state), which reduced by 0.18 ±0.09 ug.L^{-1} after 12 weeks training compared with baseline (p = 0.193) and increased by 0.21 ±0.06 ug.L^{-1} after three weeks detraining, compared with CTX-1 after 12 weeks training (p = 0.025) (Figure 31 c) In males, unlike in females, a significant effect of training state was also observed for serum P1NP (p = 0.010; η^2 = 0.438, main effect of training state). Specifically, P1NP decreased by 14.28 ±4.82 ug.L^{-1} after three weeks detraining compared with P1NP after 12 weeks DM-HIIT (p = 0.054) and was reduced significantly below baseline P1NP after detraining (116 ±55 ug.L^{-1} versus 129 ±52 ug.L^{-1}, p = 0.013; 15- versus 0- weeks, respectively) (Figure 31 d).



DM-HIIT: Diverse-Movement HIIT; TAU: Training As Usual; P1NP: N-Terminal Propeptide of Type 1 Procollagen; CTX-1: C-Terminal Telopeptide of Type I Collagen (β isoform).
* $p < 0.05$; *** $p < 0.001$.

Figure 31 Biomarkers of Bone Turnover After Training and Detraining (Mean ±SD)

8.3.9 Serum 25(OH)D

Baseline versus 12 weeks in males and in females

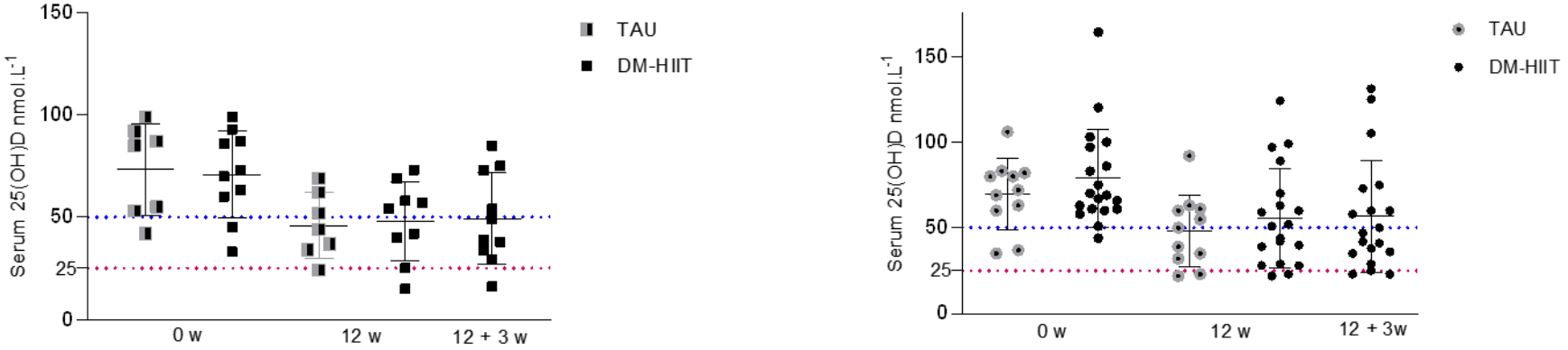
In females, serum 25(OH)D significantly reduced after 12 weeks (p < 0.001, η^2 = 0.771, main effect of time) and was 22.2 ±2.3 nmol.L^{-1} lower in December compared with in September (p < 0.001) with no difference between TAU and DM-HIIT in this decline (p = 0.399, η^2 = 0.026, time*group) (Table 35). In TAU after 12 weeks, mean serum 25(OH)D reduced below 50 nmol.L^{-1}, which has been proposed as the upper threshold for sufficiency (National Institute for Health and Care Excellence, 2020), whilst remaining sufficient in DM-HIIT (Figure 32 b). At baseline (September), serum 25(OH)D in two females (n = 1 DM-HIIT; n = 1 TAU) was between 25 - 50 nmol.L^{-1} (Figure 32 b), at which threshold treatment for insufficiency is recommended (NICE, 2020.). After 12 weeks number of participants between 25 - 50 nmol.L^{-1} increased to nine in DM-HIIT (47%) and five in TAU (45%) (Figure 32 b). In September, at entry to the study, no females were deficient (< 25 nmol.L^{-1}, NICE, 2020; ibid.) whereas in December, at the end of the intervention, two females (n = 1 TAU; n = 1 DM-HIIT) were deficient in 25(OH)D (Figure 32 b).

In males, serum 25(OH)D was also significantly lower after 12 weeks (p < 0.001, η^2 = 0.785; main effect of time) (Table 35). Mean value in both groups of males was below the upper threshold for sufficiency (NICE, 2020) (Figure 32 a). In December, in 36% of males in DM-HIIT (n = 4), and 44% in TAU (n = 4), mean serum 25(OH)D was between 25 - 50 nmol.L^{-1}, whereas at baseline it was 15% (n = 2) in DM-HIIT and 11% (n = 1) in TAU (Figure 32 b). At the end of the intervention, three participants (n = 2 DM-HIIT; n = 1 TAU) were deficient in 25(OH)D, whereas none was at baseline (Figure 32 a).

Table 35 Comparison of Serum 25(OH)D 0- versus 12-Weeks (Mean ±SD)

	Intervention Group x Timepoint				Interaction		Time		Group	
Sex	DM-HIIT		TAU		*p*	η^2	*p*	η^2	*p*	η^2
	0 w	12 w	0 w	12 w						
Females[a]	79 ±28	56 ±29[△]	70 ±21	48 ±21[△]	0.707	0.005	<0.001	0.771	0.399	0.026
Males[b]	72 ±22	48 ±19[△]	73 ±23	46 ±16[△]	0.620	0.018	<0.001	0.785	0.620	0.018

25(OH)D: Serum 25-hydroxy vitamin D (nmol.L^{-1}).[△] Significantly different from baseline ($p < 0.001$)



a Males b Females

25(OH)D: 25-hydroxy vitamin D; DM-HIIT: Diverse-Movement HIIT supplemented; TAU: Training As Usual
- - - - Sufficient; - - - - Deficient. (source: NICE, 2018).

Figure 32 Serum 25(OH)D in Male and Female Dancers at 0-, 12- and +3 Weeks Detraining (Mean ±SD)

Serum 25(OH) in DM-HIIT males and females across all training states

In DM-HIIT supplemented dancers, there was a significant effect of time on serum 25(OH)D in males ($p < 0.001$; $\eta^2 = 0.660$) and in females ($p = 0.007$; $\eta^2 = 0.601$), comparing baseline (September), after 12 weeks (December) and after three weeks detraining (January).

In females, serum 25(OH)D reduced by 23.1 ±3.2 nmol.L^{-1} after 12 weeks ($p < 0.001$) and increased by 0.9 ±2.2 nmol.L^{-1} after the detraining period ($p > 0.999$) (Figure 5 b), when it was 22.1 ±3.2 nmol.L^{-1} lower than serum 25(OH)D at baseline ($p < 0.001$) (Figure 32 b).

In DM-HIIT males, serum 25(OH)D reduced by 23.7 ±4.7 nmol.L^{-1} after 12 weeks ($p = 0.003$), showed a small increase of 2.8 ±3.2 nmol.L^{-1} after detraining ($p > 0.999$), with a trend towards a significant reduction in 25(OH)D, compared with at entry to the study ($p = 0.061$) (Figure 32 a).

The Clinical Lead at the ballet school subsequently confirmed that some dancers, who were non-UK permanent residents, returned home during the Christmas holidays, to southern hemisphere countries.

8.3.10 Gonadal steroid hormones

Baseline versus 12 weeks in males and in females

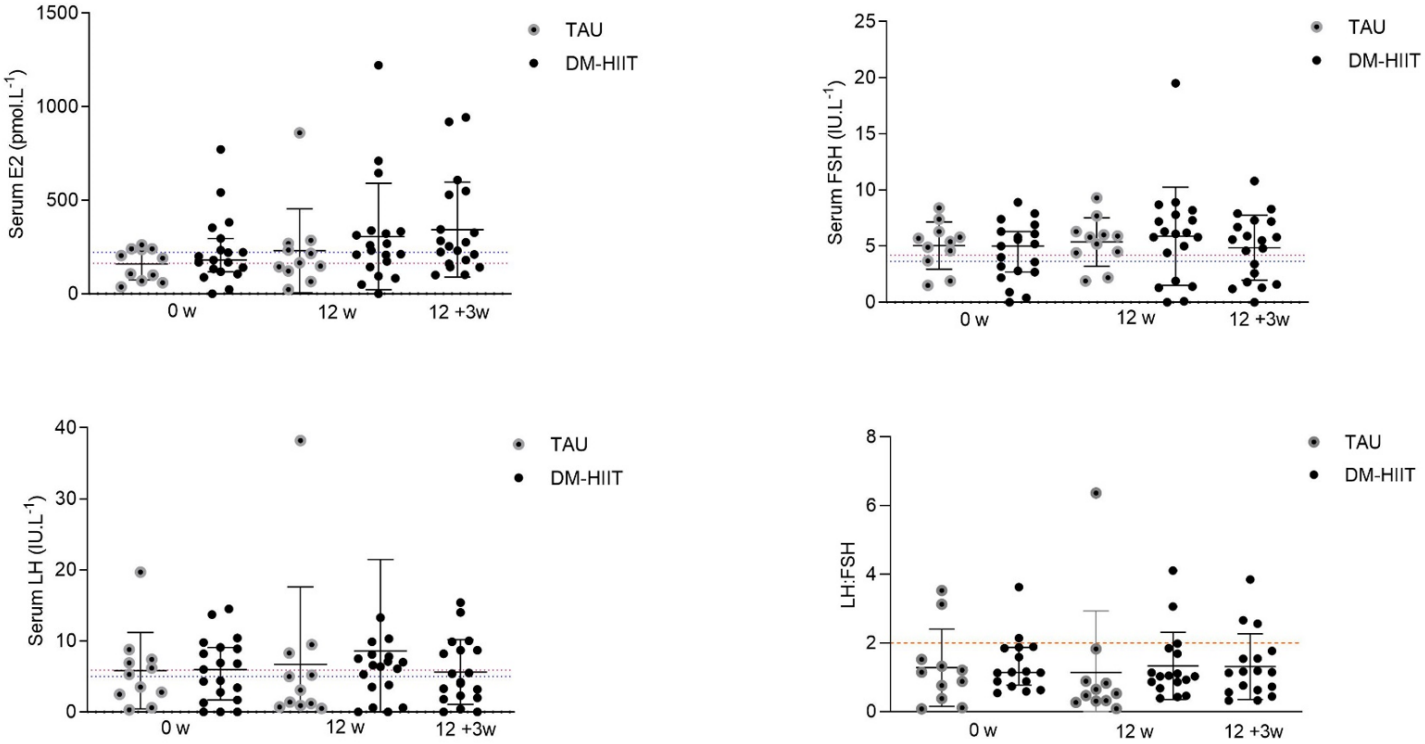
Females In DM-HIIT, E2 at all timepoints, and LH at 12 weeks were not normally distributed; in TAU, this was found for E2 and FSH at 12 weeks, for LH at baseline, and after 12 weeks. It was decided to retain the proposed statistical approach (Mixed-Model Two-Way Analysis of Variance) to compare between TAU and DM-HIIT; comparison within DM-HIIT of GnH at baseline, after 12 weeks and after 3 weeks' detraining, was undertaken using the Friedman test, with correction for multiple comparison (Dunn's).

Fasted gonadal steroid hormones (GnHs) in DM-HIIT did not differ from baseline after the intervention. Whilst sampling conditions (fasted, time of day) were controlled, it was not possible to standardise sampling time point according to participants' individual menstrual cycle phase, and data were inspected for evidence of intra-individual fluctuation, across the three gonadal hormones sampled, for indications of cyclical GnH variation. In DM-HIIT, 12 of 114 samples (7%) (data included within comparison between DM-HIIT and

TAU) had undetectable levels of GnHs, in samples provided by four individuals. Reviewing these results in relation to participant BMI at baseline, BMI was found to be slightly above the median in two of the four participants with undetectable GnHs, in one BMI was below the 25th percentile, and BMI in the fourth participant was the lowest in all female dancers (Figure 33). In the second-year cohort (TAU), no samples were below threshold for detection of estradiol (E2). Evidence of periovulatory elevation in E2, in accordance with age-specific values (Grisendi et al., 2014), was seen in seven out of a total of 60 samples (six samples in DMHIIT and one sample in TAU). These data were retained in analyses, according to the rationale that they represented valid biological variation. In DM-HIIT, 13% of DM-HIIT (five samples) and 29% of TAU (five samples) luteinising hormone (LS) was below 1 $IU.L^{-1}$ and in 13% of samples in DM-HIIT females (5 of 38) follicle stimulating hormone (FSH) was below 1 $IU.L^{-1}$. An outlier for LH at 12 weeks in DM-HIIT (59.6 $IU.L^{-1}$) was excluded from comparison of LH between female intervention groups, as the sample was above 97th percentile for 18 - 19 y reference range (Elmlinger, Kühnel, & Ranke, 2002).

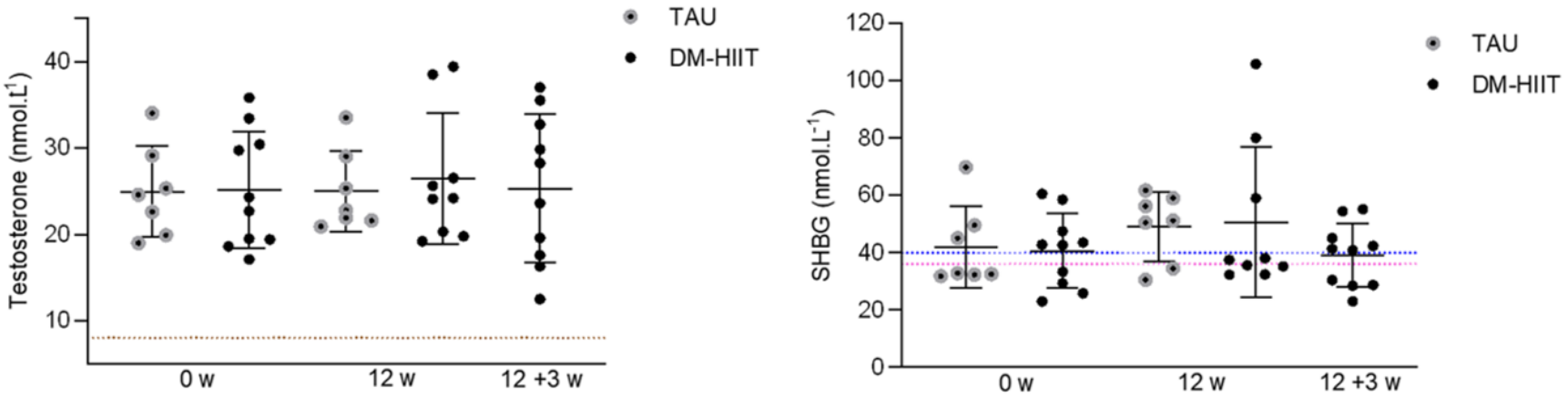
Males Sex hormone binding globulin (SHBG) significantly increased after 12 weeks by 8.9 ±3.7 $nmol.L^{-1}$ ($p = 0.028$; $\eta^2 = 0.299$; main effect of time), no differences in total testosterone were observed.

In one DM-HIIT male participant, SHBG was elevated (2 SDs above mean) at 12 weeks, relative to cohort values (Figure 34). Data were checked for errors and after none was identified, other data provided by the participant were inspected. BMI was found to be below the threshold for underweight (18.5 $kg.m^2$, source: www.nice.org.uk) and at the time of sampling, BMI in this participant was the lowest in DM-HIIT males. Relative to cohort values, CTX-1 in the participant was low after 12 weeks training (below 25th percentile), however, P1NP was above the 50th percentile in DM-HIIT males.



E2: Estradiol; FSH: Follicle stimulating hormone; SH: Luteinising hormone; TAU: Training as usual; DM-HIIT: Diverse-Movement HIIT; - - - - Denotes 18 – 19 y reference value (Elmlinger et al., 2002); - - - - Denotes median percentile 17 y reference value (Elmlinger et al., 2002; ibid.); - - - - Denotes ~threshold for elevation in LH:FDH ratio
Note. Outlier for LH at 12 w in one participant in DM-HIIT (59.6 $IU.L^{-1}$) not plotted for reasons of scale.

Figure 33 Fasted Gonadal Steroid Hormones in Female Dancers (Median IQR)



T: Total Testosterone; SHBG: Sex Hormone Binding Globulin; TAU: Training As Usual; DM-HIIT:Diverse-Movement HIIT.
- - - - Denotes median percentile 18-19 y reference value (Elmlinger et al., 2002); - - - - Denotes median percentile 17 y reference value (Elmlinger et al., ibid.); . . . . Denotes threshold for Testosterone sufficiency (British Society For Sexual Medicine, January 2018; https://www.guidelines.co.uk/).

Figure 34 Fasted Gonadal Steroid Hormones in Male Dancers (Mean ±SD)

Gonadal steroid hormones after detraining in DM-HIIT

In DM-HIIT females, fasted serum E2, LH and FSH did not vary significantly across sampling timepoints (0 weeks, 12 weeks, 15 weeks) (Table 36).

In DM-HIIT males, whereas T did not vary across sampling timepoints, there was a tendency for SHBG to increase after 12 weeks training (Table 36), (p = 0.075; η^2 = 0.340; main effect of time). In the DM-HIIT participant with elevated SHBG and testosterone at 12 weeks, detraining values for these hormones remained high (above 75th percentile), relative to the cohort, but were not outliers.

Table 36 Gonadal Steroid Hormones in DM-HIIT

	DM-HIIT Females (n = 19)⌂			One-way ANOVA		Friedman
Hormone	0 w	12 w	15 w	p	η^2	p
E2 pmol.L^{-1}	180 (118, 295)	232 (143,333)	254 (160, 943)	- -	- -	0.368
FSH IU.L^{-1}	5.0 (2.7, 6.3)	6.1 (1.9, 7.8)	5.5 (1.8, 7.2)	- -	- -	0.776
LH IU.L^{-1}	1.7 (6.0, 9.1)	6.6 (3.5, 8.1)	4.2 (2.0, 8.7)	- -	- -	0.774
	DM-HIIT Males (n = 11)					
T nmol.L^{-1}	25.4 ±7.1	26.4 ±7.6	25.5 ±9.1	0.710	0.042	- -
SHBG nmol.L^{-1}	40.6 ±12.9	50.6 ±26.1	38.7 ±11.7	0.075	0.340	- -

E2: Estradiol; FSH: Follicle Stimulating Hormone; LH: Luteinising Hormone; T: Total Testosterone; SHBG: Sex Hormone Binding Globulin.
Note. ⌂ Outlier data (n = 1) for LH at 12 w, n = 18 used for LH comparison.
E2, FSH, LH: Median (IQR); T, SHBG: Mean (±SD).

8.3.11 Cardiovascular measures and body composition

Heart rate and blood pressure

In DM-HIIT females, heart rate (HR) at rest was significantly reduced after 12 weeks compared with TAU (p = 0.026; η^2 = 0.273; time by group interaction), decreasing from 82 ±10 to 69 ±12 bpm (p < 0.001), compared with 76 ±14 to 73 ±12 bpm (p = 0.850) in TAU. Resting systolic and diastolic blood pressure (BP) and mean arteriral pressure (MAP) did not change significantly in either group.

In males, no changes in resting HR, systolic or diastolic BP or MAP were observed after 12 weeks.

8.3.12 Anthropometrics

Height, body weight and BMI

Mean height did not change in any group after the intervention.

In females, body weight significantly increased after 12 weeks (p = 0.004; η^2 = 0.228, main effect of time) by 1.1 ±0.4 kg (p = 0.004) and BMI also increased (p = 0.005; η^2 = 0.225, main effect of time) by 0.45 ±0.15 kg.m^{-2} (p = 0.005) (Figure 35), with no difference found between groups (Table 37).Whilst mean values at baseline in DM-HIIT and TAU females met the threshold for 'healthy weight' (18.5-24.9 kg.m^{-2}), according to current BMI guidelines (NICE, 2014), BMI in 5 participants in DM-HIIT (26%), and in 7 participants in TAU (45%) was below 18.5 kg.m^{-2} (Figure 35).

In males, there were no significant main or interaction effects for BMI (Figure 35) or body weight (Table 37). No males at baseline exhibited low BMI, however, after 12 weeks in two males (n = 1 DM-HIIT; n = 1 TAU) BMI was below 18.5 kg.m^{-2} (Figure 35).

In TAU, three females and one male, and in DM-HIIT two males, were unavailable at 12 weeks for anthropometric data collection. Results are presented from analysis of data provided at baseline and after 12 weeks.

Hip and waist circumference

Females HC in DM-HIIT was significantly different from TAU after 12 weeks training (p = 0.042, η^2 = 0.123, time*group) and reduced in DM-HIIT by

3.4 ±4.8 cm (p = 0.002) compared with a reduction of 0.4 ±3.1 cm in TAU (p > 0.999) (Table 37). WC after 12 weeks training was also significantly different between intervention groups (p = 0.012, η^2 = 0.180, time*group). WC reduced by 3.2 ±3.6 cm in DM-HIIT females (p < 0.001) and by 0.6 ±1.8 (p = 0.912) in TAU. No significant changes in waist to hip ratio (W:H) were observed.

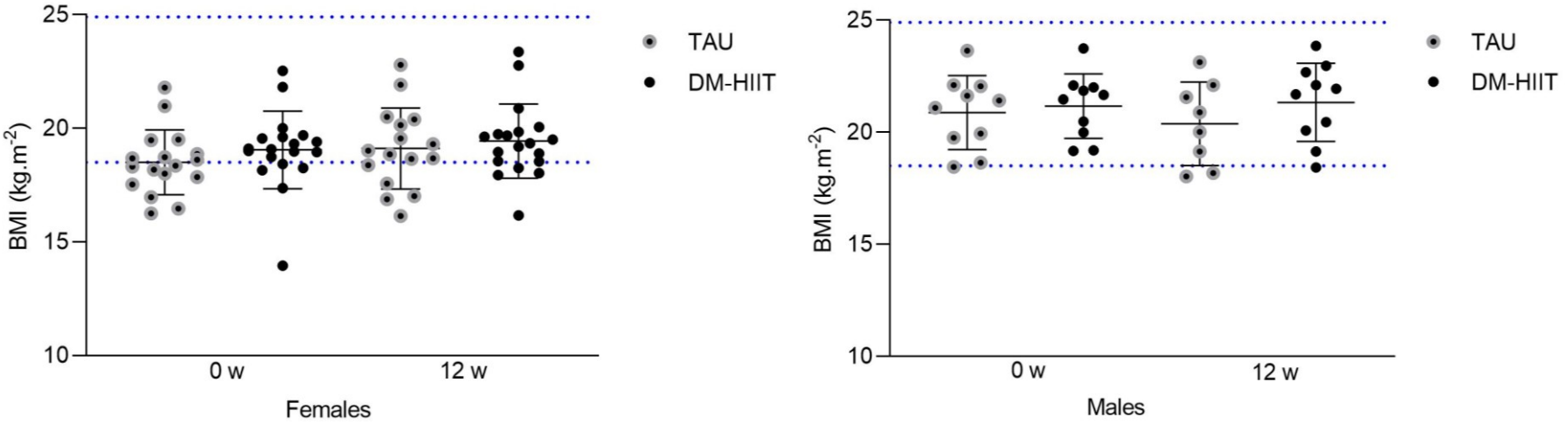
In males, as in females, HC was significantly different in DM-HIIT compared with TAU after 12 weeks training (p = 0.013, η^2 = 0.364, group*time). HC reduced by 4.3 ±3.0 cm in DM-HIIT compared with baseline (p < 0.001) and reduced in TAU by 0.6 ±2.0 cm (p = 0.970) (Table 37). Unlike in females, there was no difference in WC between groups after 12 weeks training (p = 0.289, η^2 = 0.080, group*time, WC). WC reduced in both groups after 12 weeks (p < 0.001, η^2 = 0.603, main effect of time) by 2.9 ±0.6 cm, compared with baseline (p < 0.001, pairwise comparison). As observed in females, W:H did not change.

Table 37 Anthropometric Data in Males and Females 0- Versus 12- Weeks (Mean ±SD)

Females	DM-HIIT (*n* = 19)		TAU (*n* = 15)		Interaction		Time		Group	
Measure	0 w	12 w	0 w	12 w	*p*	η^2	*p*	η^2	*p*	η^2
Weight kg	53.1 ±5.6	53.9 ±5.9	49.9 ±4.6	51.3 ±4.8	0.451	0.018	0.004	0.228	0.119	0.074
Waist cm	68.0 ±5.3	64.7 ±4.0**	64.6 ±3.1	64.0 ±2.8	0.012	0.180	0.001	0.309	0.117	0.075
Hip cm	85.9 ±5.9	82.5 ±4.9***	82.6 ±4.6	82.2 ±4.6	0.042	0.123	0.012	0.183	0.270	0.038
WHR AU	0.79 ±0.04	0.79 ±0.05	0.78 ±0.03	0.78 ±0.03	0.843	0.001	0.534	0.012	0.517	0.013

Males	DM-HIIT (*n* = 8)		TAU (*n* = 8)							
	0 w	12 w	0 w	12 w						
Weight kg	66.8 ±2.2	67.1 ±2.3	62.0 ±2.4	61.8 ±2.5	0.602	0.017	0.904	0.001	0.145	0.128
Waist cm	75.9 ±5.0	72.3 ±4.0	73.6 ±4.0	71.3 ±3.9	0.289	0.080	<0.001	0.603	0.433	0.044
Hip cm	90.7 ±5.0	86.4 ±6.9***	85.1 ±6.1	84.5 ±6.7	0.013	0.364	0.002	0.514	0.251	0.093
WHR AU	0.84 ±0.02	0.84 ±0.02	0.87 ±0.05	0.85 ±0.06	0.301	0.076	0.372	0.057	0.329	0.068

DM-HIIT: Diverse-Movement HIIT; TAU: Training As Usual.
Note. WHR: Waist to Hip Ratio; AU: Arbitrary Units.
* Significantly different after 12 weeks training ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$).



DM-HIIT:Diverse-Movement HIIT; TAU: Training As Usual.
- - - - Denotes lower and upper thresholds for 'healthy BMI' (source: NICE, 2014).

Figure 35 BMI in Male and Female Ballet Dancers (Mean ±SD)

Table 38 Heart Rate During Submaximal Exercise 0- Versus 12-Weeks (Mean ±SD)

		DM-HIIT (*n* = 15)		TAU (*n* = 9)		Interaction		Time		Group	
Sex	Measure	0 w	12 w	0 w	12 w	*p*	η^2				
F	HR_{MEAN}	140 ±8	140 ±10	148 ±9	145 ±14	0.582	0.014	0.567	0.015	0.036	0.184
F	HR_{MAX}	169 ±10	167 ±14	172 ±10	166 ±13	0.444	0.027	0.166	0.085	0.776	0.004
		DM-HIIT (*n* =8)		TAU (*n* = 5)							
M	HR_{MEAN}	142 ±14	134 ±11	148 ±9	144 ±13	0.429	0.058	0.053	0.298	0.196	0.197
M	HR_{MAX}	166 ±11	155 ±12	168 ±10	159 ±11	0.764	0.009	0.001	0.625	0.581	0.029
M	HR_{MIN}	97 ±17	89 ±10	92 ±11	98 ±16	0.011	0.455	0.252	0.117	0.198	0.146

DM-HIIT: Diverse-Movement HIIT; TAU: Training As Usual; HR: Heart Rate.

8.3.13 Physiological responses to submaximal exercise

Capillary blood lactate concentration

In females, capillary blood lactate (BLa) concentration immediately after submaximal exercise reduced after 12 weeks (p = 0.047; η^2 = 0.138, main effect of time) by 1.0 ±0.5 mmol.L^{-1} (p = 0.047), from 4.1 ±1.7 to 3.0 ±1.7 mmol.L^{-1} in DM-HIIT and from 4.3 ±2.8 to 3.4 ±2.6 mmol.L^{-1} in TAU. No effect of interventional group was found (p = 0.631; η^2 = 0.009, main effect of group).

In males, an inverse direction of effect was observed: BLa concentration increased after submaximal exercise after 12 weeks training (p = 0.043; η^2 = 0.279; main effect of time) by 1.5 ±0.7 mmol.L^{-1} (p = 0.043). In DM-HIIT males BLa concentration increased from 2.3 ±0.9 to 4.0 ±2.1 mmol.L^{-1}, and in TAU males from 3.7 ±1.5 to 5.0 ±2.6 mmol.L^{-1}.

Heart rate

Females Mean heart rate during submaximal exercise was 7 ±3 bpm lower in DM-HIIT than TAU (p = 0.036) (Table 38). Minimum heart rate in females was not analysed due to drop out of the heart rate trace in several participants.

Males Mean heart rate tended to be lower during submaximal exercise after 12 weeks by 6 ±3 bpm (p = 0.053) and maximum heart rate reduced by 9 ±2 bpm (p = 0.001) (Table 38). Minimum heart rate differed between groups after 12 weeks training (p = 0.011; η^2 = 0.455, time* group interaction effect), and increased in TAU (111 ±15 versus 92 ±11 bpm, p = 0.041) but not in DM-HIIT (89 ±10 versus 97 ±17 bpm, p = 0.341).

Locomotor profile during submaximal exercise

Females After 12 weeks lateral accelerations differed between DM-HIIT and TAU (p = 0.032, η^2 = 0.150, main effect of group, ‘sideways’). In TAU duration increased by 0.2 ±0.1 s in TAU after 12 weeks (p = 0.032) but was unchanged in DM-HIIT. Above 2 g duration decreased after 12 weeks (p = 0.001, η^2 = 0.328, main effect of time) by 0.1 ±0.0 s (p = 0.001, pairwise comparison).

DM-HIIT tended to be different in angular acceleration profile after 12 weeks training (p = 0.053, η^2 = 0.098, group*time*angular plane). DM-HIIT

females exhibited 1.6 ±0.7 s less axial (left-right) trunk acceleration (p = 0.049), whereas there was no change in TAU.

Males DM-HIIT and TAU differed in duration of vertical accelerations after 12 weeks training (p = 0.042, η^2 = 0.261, group*time*gband, within 'up'). In DM-HIIT duration was 1.6 ±0.6 s lower between 1 - 1.5 g (p = 0.045) compared with TAU males.

Duration of angular accelerations reduced in DM-HIIT in the frontal plane ('yaw') between 30 and 60 degrees (p = 0.015, η^2 = 0.354, time*group, 30 - 60 RBand) and between 60 and 90 degrees (p = 0.005, η^2 = 0.444, time*group 60 - 90 RBand) compared with baseline but did not change in TAU.

8.3.14 Qualitative responses and feedback

Participant evaluation of supplemental DM-HIIT

Responses to the VAS used to assess participants' perception of the effect of DM-HIIT showed that females rated DM-HIIT participation as having provided 66 ±17% improvement on dance fitness and males rating was 74 ±11%.

Teaching staff

No formal feedback process with staff was implemented, however, anecdotal comments volunteered from three ballet teachers were: 'dancers are leaner'; 'dancers have improved physique'; 'technical stamina has improved'.

The Head of Medical services requested that the protocol be retained, so that it could be implemented for the subsequent third year group.

8.4 Discussion

Results from this study in vocational ballet dancers suggest that a low volume of supplemental diverse exercise reduced background bone resorption in female dancers, however, there was no evidence it provided a stimulus to bone formation, which was speculated to reflect attenuating effects of hypothalamic dysregulation detected in females' gonadal steroid hormone profile. A detraining effect, characterised by increased bone resorption in females and in males who received diverse HIIT, was observed after three weeks and in male dancers' bone formation also increased. No effect of 12 weeks diverse HIIT on calcaneal BUA was observed.

In female dancers, who supplemented habitual ballet training with twice weekly bouts of brief (12.5 min) , diverse HIIT, bone resorption, characterised by CTX-1 at rest, significantly reduced after 12 weeks but did not change in females who trained as usual. In males, bone resorption also decreased after 12 weeks training, however, no effect of superimposition of diverse HIIT was seen.

Alongside these effects in bone turnover markers (BTMs), acceleration profile during diverse-movement (DM)-HIIT demonstrated a direction-specific adaptation to chronic training occurred in both male and female dancers. However, results suggested that the protocol was a much bigger divergence from usual training in females than in males. Whereas at the lowest threshold (1 – 1.5 g) duration of vertical accelerations increased by 16.1 ±17.1 s in males after 12 weeks training, in females an increase at this threshold of 21.6 ±3.0 s was seen. Moreover, only in females was the increase in duration at the highest threshold (> 3 g) significant compared with baseline ($p < 0.001$). According to the premise that acceleration profile is a proxy to estimate mechanical loading during exercise (Jämsä et al., 2011), this finding suggests that in female dancers loading intensity, characterised by an increase in higher threshold vertical accelerations, was greater after 12 weeks training. Furthermore, accelerations at higher thresholds (> 3.9 g) are proposed to be an indicator of osteogenic effects of exercise in premenopausal women (Vainionpää et al., 2006), and increased hip and femoral BMD is positively associated with daily exposure to accelerations > 4 g during habitual physical activity (PA). It may be speculated on the basis of this evidence that the reduction in CTX-1 in DM-HIIT

females, in the absence of any evidence of upregulation in bone formation, represents a net pro-bone response to high-intensity mechanical loading experienced during DM-HIIT.

Methodological differences between the present study, and others that have examined the relationship between acceleration profile, daily PA and bone responses, hampers comparison of present results with those of others that have adopted a similar approach. For example, Vainionpää and colleagues metered the recommended acceleration dose in counts (100 daily above 3 g) as did Jämsä et al., (60 daily above 4 g) rather than durations, as applied here. At a sampling rate of 100Hz.s^{-1} for the movement sensors used during DM-HIIT, length of exposure time for high-threshold accelerations (1.0 ±0.2 s), derived in counts from the time-integrated accelerometric signal, represented a ~100 count increase after 12 weeks training, and is in alignment with what has been proposed to elicit an osteogenic effect in premenopausal women (Jämsä et al., 2006). High-threshold (> 3g) accelerations, representing for the present protocol the intensity frequency hypothesised to be osteogenic, were most likely generated by vigorous exercise efforts. Therefore, according to the original (Frost, 1987) and revised (Tyrovola & Odont, 2015) mechanostat, it was hypothesised that unusual actions, more commonly experienced by males during floor work, were promotors of an osteogenic response, as a result of exposure to higher magnitude and more diverse mechanical strains than those prevailing during habitual ballet training.

This is somewhat supported by evidence of a sex-based divergence in indices of cardiorespiratory fitness in professional ballet dancers. Superior VO_{2MAX}, higher maximum heart rate and greater energy expenditure have been demonstrated in males during training and rehearsal, compared with females (Beck, Redding, & Wyon, 2015; Cohen, Segal, & McArdle, 1982), and usual training activities in males, particularly principals, impose higher demands for explosive actions and aerobic power (Koutedakis & Jamurtas, 2004). As an explanation for present findings in female dancers, however, the hypothesis that unusual high-intensity mechanical strains encountered during DM-HIIT were the driver for constitutive downregulation of resorption, relies on an assumption of underlying continuity between proxy indicators of training adaptation and BTMs. Nevertheless, activities featured in the protocol were chosen to provide

opportunities for vertical and lateral impacts and multidirectional accelerations, and female dancers were encouraged to brake hard at the edge of the exercise area and slam through the foot during landing, which they are not accustomed to in usual technique (Liiv et al., 2013).

The exercise movements applied during DM-HIIT were harvested from team sports, and included 20 s of medium-intensity (MI) and 10 s high-intensity (HI) vertical and lateral jumping, sagittal shuttle runs and progressive bounding and hopping. In addition, as exercise area was limited (1.5 x 3 m) whole-body deceleration and acceleration were imposed at high-density during change of direction (COD) at the edges of the floor grid. Similar actions in team sports are speculated to provide the stimulus to musculoskeletal adaptation, including whole-body and regional increases in BMD, and upregulation in biomarkers of bone formation, in females who train with football (Jackman et al., 2013; Krustrup, Helge, et al., 2018), particularly in a restricted pitch size, which increases the frequency of high-intensity actions and COD (Randers et al., 2010). Interestingly, a similar locomotor profile of high-impact jumping, diagonal traversing and rapidly accelerated (COD) has been characterised in time-motion and work-load analysis in ballet (Twitchett et al., 2009; Wyon et al., 2007), and typifies artistic performance demands on male soloists. This tends to support the hypothesis that activities prescribed during DM-HIIT were more unusual for female ballet dancers than males, and therefore could explain the difference in adaptation to high threshold accelerations, which was significant in females but not males, presumably as females adapted from a more naïve movement capacity in the basal state.

Moreover, as background BTMs did not change in DM-HIIT males, unlike in females, and were not significantly different from TAU males, this further supports the hypothesis that the mechanical stimulus provided by diverse HIIT exceeded mechanical signals prevailing in habitual female ballet training, but did not exceed prevailing loading in males. According to this proposal, detraining BTM responses in males could be interpreted as representing the effect of withdrawal from the stimulus to bone metabolism provided by usual training, and this would explain the significant decrease in bone formation and increase in bone resorption observed in DM-HIIT males on return from the Christmas break. In contrast, lack of change in P1NP in DM-HIIT females after

detraining could indicate that usual exercise did not provide a sufficiently osteogenic stimulus for withdrawal to result in downregulation of formation. Accordingly therefore, the increase in CTX-1 in females after the Christmas break could indicate that withdrawal from supplemental diverse HIIT was permissive for resorption.

Despite a proposal of net pro-bone influence for the supplemental exercise, biomarker responses in females who received diverse-movement HIIT (DM-HIIT), specifically 9.4 ±22.9% reduction in serum CTX-1, alongside no change in P1NP, are contrary to what is characteristically reported in accordance with osteogenic effects of exercise characterised by bone turnover markers (BTMs) (Dolan et al., 2020). An initial increase in resorption, proposed to initiate osteoblast activity (Hadjidakis & Androulakis, 2006), is typically followed by enhanced formation in the longer-term (Dolan et al., 2020) and results in bone accrual. No changes were observed in calcaneal QUS in either DM-HIIT females or females who trained as usual (TAU), which is in accordance with the evidence that background bone formation was not increased. Alternatively, this could reflect biological and ecological factors, such as gonadal hormone and serum 25(OH)D status, acting independently and in concert to mitigate the exercise signal, attenuating the potential for an osteogenic effect promoted by elevation in P1NP.

Gonadal steroid hormone (GnH) profile showed significant hypothalamic-pituitary-axis (HPA) perturbation within a sub-group of female dancers, and was indicative of functional hypthalmic amenorrhoea (FHA), a clinical entity in which luteinising hormone (LH) and follicle stimulating hormone (FSH) are dysregulated. Nevertheless, calcaneal bone parameters in female dancers were above age-referent values, suggesting that long-term dance training had provided a stimulus to osteogenesis. It is possible to reconcile evidence of MD with adequate calcaneal bone as menstrual disturbance in athletes is demonstrated to fluctuate in severity, and can reflect recent or even daily trends in energy balance (Williams et al., 2015). Therefore, although a hypooestrogenic state, defined by undetectable serum E2 or E2 below age-referent 25th percentile (Elmlinger et al., 2002) was observed in a sub-group of six participants (*n* = 4 DM-HIIT; *n* = 2 TAU), and elevated FH:LSH (≥ 2) with normal E2 was detected in a further sub-group of five participants (*n* = 4 DM-

HIIT; *n* = 1 TAU), it is possible that these were phenomena of relatively recent duration, and indicative of altered eating behaviours, for example in response to competitive pressure to succeed in forthcoming professional auditions.

In the present study, dietary intake was not monitored, in agreement with stakeholders; and therefore energy availability (EA) cannot be estimated. However at baseline, low BMI (< 18.5 $kg.m^2$) in 14 females (*n* = 5 DM-HIIT; *n* = 9 TAU) alongside levels self-reported amenorrhoea (*n* = 2 DM-HIIT; *n* = 2 TAU), irregular menses (*n* = 5 DM-HIIT; *n* = 5 TAU) and non-commencement of menses (*n* = 1 DM-HIIT; *n* = 1 TAU), provided further ecological evidence of menstrual dysfunction and somatotypical leanness. It can only be speculated that these findings are indicative of MD associated with REDS, as metabolic profile was not characterised, for example, by measuring leptin and ghrelin, shown to be paradoxically reduced and elevated in adolescent amenorrheic athletes, compared with eumenorrheic athletes and controls (Ackerman et al., 2012).

Although bone metabolism indicated a reduction in resorption in DM-HIIT females, as was cautioned in a recent publication, claiming an osteogenic effect for an intervention justified by dichotomous evidence of increased/decreased/formation/resorption is somewhat meaningless, without an ecological context against which magnitude, direction and dynamics of reported BTM changes may be assessed (Dolan et al., 2020). For example, CTX-1 is typically (and predictably) reduced by ~60% after 3 - 6 months pharmacological intervention in postmenopausal osteoporosis (Garnero, 2017), whereas in DM-HIIT females, magnitude of reduction in CTX-1 after 12 weeks training (9.4 ±22.9%), and increase (10 ±11%) after three weeks detraining, were much lower. Present findings are more comprehensible in the context of evidence from younger, healthy populations, as recommended for interpreting bone biomarkers in premenopausal females outside a clinical scenario (Jain & Camacho, 2018).

Compared with background serum BTM reference intervals in young Australian women, at baseline CTX-1 in DM-HIIT (0.79 ±0.25 $ug.L^{-1}$) and TAU females (0.78 ±0.23 $ug.L^{-1}$), and after twelve weeks in TAU (0.80 ±0.28 $ug.L^{-1}$), were at the upper end of the interquartile range (0.56 – 0.81 $ug.L^{-1}$) for females aged 16-19 y (Callegari, Gorelik, Garland, Chiang, & Wark, 2017). However,

after 12 weeks training, mean CTX-1 in DM-HIIT females aligned closely with the median (0.72 ug.L^{-1}) referent value for this age group (Callegari et al., 2017). In an acute investigation in young (22.5 y) recreationally active females, basal CTX-1 prior to exercise was also lower (0.5 ug.L^{-1} [derived from graphical results]) than mean values in DM-HIIT and TAU at all sampling timepoints (Kouvelioti et al., 2018). In an observational study in professional ballet dancers, which examined seasonal effects of vitamin D, CTX-1 was lower in ballerinas using oral contraception (OCU) (0.5 ±0.09 ug.L-1, [sic]) than in DM-HIIT or TAU, and mean in non-OCU (0.7 ±0.31 ug.L^{-1}, [*sic*]) could not be compared due to reporting style (1 dp vs 2 dp in the present study) (Wolman et al., 2013). Although the latter biomarker study is comparatively unusual in the literature, as it directly accessed data from dancers within the same aesthetic athlete population as DM-HIIT and TAU, comparison with present findings is problematic, as sample size was smaller (*n* = 13), the ballerinas were ~6 y older and at professional rather than vocational level, and OCU use was higher (38%) (Wolman et al., 2013), than in DM-HIIT (26%) and TAU (19%).

In the context of reference data, and evidence from healthy, similarly aged female populations, the reduction in sCTX-1 in DM-HIIT females after supplemental exercise could be interpreted as a biologically meaningful decrease in bone resorption biomarker status, towards alignment with values representative of healthy young females, in response to diverse HIIT. Moreover, this effect was not seen in TAU, who otherwise trained at the same volume (35 h per week) as DM-HIIT. However, as the least significant change (LSC) for CTX-1 between two measurements in an individual is 0.08 ug.L^{-1}, the magnitude of change in resorption (0.08 ±0.03 ug.L^{-1}) is at the threshold of significance for a result exceeding background variability inherent in the sampling method (Jain & Camacho, 2018). Furthermore, whereas it is plausible that reduction in resorption could in the longer-term have a net pro-bone effect, by favouring background bone formation, we did not observe a constitutive increase in P1NP, and therefore a direct osteogenic effect for DM-HIIT in females at the dose applied is not supported by present findings.

In male dancers, background serum CTX-1 and P1NP sampled at rest were higher than in females, although statistical comparison was not performed. There is evidence of an earlier decrease in BTMs in females (Szulc, 2018), and

a late-occurring growth spurt in males is consistent with higher BTMs, reflecting sustained linear growth and bone modelling, and this is supported by age-relevant population data for sBTMs (Rauchenzauner et al., 2007). Mean serum P1NP was higher across the intervention in TAU males compared with DM-HIIT (252 ±37 ug.L^{-1} versus 130 ±33 ug.L^{-1}), who were on average a year older (18.4 ±0.8 y versus 17.5 ±0.8 y) than TAU. As bone formation is demonstrated to peak between 12 - 14 y, according to biomarker evidence in young males (Matsukura et al., 2003; Tommasi et al., 1996), P1NP in TAU may be speculated to indicate a delay in maturation in this cohort. It is unlikely that difference in training loads from background dance practice during the intervention phase could account for significantly higher BTMs in TAU, as these were closely aligned between groups (35 h per week), and therefore osteogenic effects of non-HIIT exercise exposure were matched by convenience. Therefore, it is more likely to be attributable to delayed maturation and ongoing bone modelling activity in TAU males. This is somewhat supported by the significant bilateral increase in calcaneal speed of sound (SOS) in males, (left: 6.22 ±2.76m.s^{-1}; right: 9.38 ±4.20m.s^{-1}) (data pooled), which was not different between DM-HIIT and TAU, and exceeded long-term precision (3.14 – 5.5%) for SOS measured by QUS (Njeh et al., 2000). As this parameter is considered to reflect elastic and compressive material properties (Cavani et al., 2008) and bone microarchitecture (Hans & Baim, 2017; Padilla & Laugier, 2005), and as ground reaction force is less attenuated at the heel, results are likely to indicate a true, mechanically elicited response in males to impacts from landing and jumping,

Total serum testosterone (T) and sex hormone binding globulin (SHBG) were within age-referent values (Elmlinger et al., 2002), and whilst T was unchanged, SHBG increased after 12 weeks from 40.6 ±12.9 to 50.6 ± 26.1 nmol. L^{-1} (24 ±28%) in DM-HIIT males, and from 38.9 ±13.4 to 49.0 ±12.1 nmol.L^{-1} (22 ±33%) in TAU. Although it is difficult to interpret this finding mechanistically, there is a proposed regulatory role for SHBG in age-related bone loss (Kim, Koo, et al., 2017), together with evidence of strong, positive correlation between SHBG and elevations in serum bone turnover markers (β-xCTX and P1NP) in older (~59 y) men (Boonen et al., 2011). Therefore, elevation in SHBG in male dancers may reflect an increase in remodelling, in accordance with bone metabolic responses to training and an osteogenic effect

of male dancers' activities. Additionally, as increased central adiposity is associated with reduced SHBG and greater insulin resistance, the elevation observed in SHBG may indicate a training-induced change in insulin sensitivity and energy balance, alongside inclination towards leanness. This is partly supported by single case evidence of high (> 2 SDs above mean) SHBG in one male who exhibited the lowest BMI value (18.43 $kg.m^2$) after 12 weeks in DM-HIIT. Fewer males in both year groups limited examination of possible sub-groups, however results suggest that characterising T3 and insulin status, alongside regulatory biomarkers of energy balance, such as ghrelin and leptin, would provide comprehensive insight into EA in male dancers, and its relationship with biomarkers of bone metabolism and gonadal steroids.

There was a highly significant seasonal effect on serum 25-hydroxyvitamin D (25(OH)D) in all groups: in females 25(OH)D decreased from 75.5 ±25.9 to 53.0 ±26.1 $nmol.L^{-1}$, and in males decreased from 72.4 ±21.7 $mmol.\ L^{-1}$ to 47.2 ±17.4 $nmol.L^{-1}$ after twelve weeks (data pooled within sex). It may be speculated that adaptation in bone and muscle to the training stimuli of DM-HIIT, and usual dance practice, could have been attenuated, in accordance with evidence of impaired adaptation in athletes associated with compromised 25(OH)D status (Hamilton, 2010), although mechanisms underlying its pleiotropic effects, and reference analyte, are controversial (Książek, Zagrodna, & Słowińska-Lisowska, 2019). In ballet dancers, significant increases in jump height, and lower soft tissue injuries were seen in a cohort who received oral supplementation with vitamin D, compared with dancers who did not (Wyon, Koutedakis, Wolman, Nevill, & Allen, 2014). In the light of this evidence, it is possible that capacity to perform explosive jumping could have been impaired in dancers in DM-HIIT and TAU who were insufficient or deficient after 12 weeks, and correspondingly, magnitude of impacts, and associated attenuation of compression effected by lower limb muscle contractions, would have reduced. Despite the finding of a seasonal reduction, no association between 25(OH)D and calcaneal QUS, or markers of bone metabolism was found. Although the time frame is likely to have been too brief for a potential catabolic response in calcaneal bone to be detected, QUS may have been inadequate to resolve this, given longer-term precision is 3.14 – 5.5% for repeated measurement of broadband ultrasound attenuation (BUA) within an individual (Njeh et al., 2000).

In other studies that similarly observed a seasonal reduction in 25(OH)D, in female indoor athletes (Maruyama-Nagao, Sakuraba, & Suzuki, 2016), and in male and female professional ballet dancers (Wolman et al., 2013), an inverse relationship with parathyroid hormone (PTH) was reported. Mechanistically, PTH is proposed to increase bioavailability of calcium in the presence of low serum vitamin D (Fleet, 2017). In the present study, whilst no relationship between reported daily calcium intake and serum 25(OH)D or calcaneal QUS was found, dancers indicated high consumption of milk products, and exceeded daily RDA (700 $mg.day^{-1}$) (NICE, 2018 [3]) in all but six participants (*n* = 1 male; *n* = 5 females). It would seem that in these dance cohorts, dietary habits offset the risk for an acute elevation in PTH in association with calcium defence during exercise exposure (Kohrt et al., 2018), and for a constitutive increase in response to reduced serum 25(OH)D (Maruyama-Nagao et al., 2016).

Whilst key outcome measures in the present study were bone related, there was indication of a relative training effect on dancers' fitness to perform intermittent actions prescribed during HIIT after 12 weeks DM-HIIT supplementation. Whereas duration of vertical accelerations across all thresholds increased by 34.6 ±19.6 s in females and 25.3 ±25.0 s in males during 485 s of dynamic actions, maximum and mean exercise heart rate reduced, and there was no change in venous BLa concentration immediately, or two minutes, after exercise. As acceleration profile showed workload increased, metabolic cost would be greater, yet non-oxidative contribution to metabolism was not correspondingly elevated, and cardioperturbation was less, which suggests an adaptation in dancers' capacity for intermittent high-intensity actions during the protocol. No formal assessment of aerobic fitness was undertaken, however qualitative evidence supported a perception of increased fitness. Dancers' rated demand on breathing as significantly reduced after 12 weeks training, but not the demand on legs, which is consistent with higher acceleration workload at lower cardioperturbation after 12 weeks training.

Dance-specific fitness testing in ballet students has shown high reliance on skill alongside low aerobic capacity (Wyon, Redding, Abt, Head, & Sharp, 2003), despite the identified requirement for aerobic power to perform at intermittence, which characterises elite ballet performance (Beck et al., 2015). As no adverse event or injuries were associated with training, and compliance

with DM-HIIT was 91 ±10% in females and 75 ±20% in males, the regimen could provide an acceptable and time-efficient way for dancers to enhance capacity to exercise at intermittent high-intensity, providing an ecologically relevant adjunct to usual training.

8.4.1 Limitations

There are several limitations to the present investigation. We used convenience sampling, in which second year students continued usual dance training, rather than a control group, and as dancing training load was not controlled, it cannot be excluded this was different between the two year groups. The duration of the study (12 weeks) was dictated by term length and training schedule (2 x weekly exercise) by availability in the dancers' timetable. Longer (> 16 weeks) and additional (1 - 2) weekly bouts would have been more appropriate for bone adaptation to be amenable to quantification.

A sub-group of females in TAU (*n* = 7) declined venipuncture, and there were more females than males in each year group, which affected effective matching for some comparisons. In consultation with staff, it was agreed that data gathering around body composition and menstrual status would be minimally intrusive, and therefore follow up on declared MD was not possible.

With regard to methods, it was not acceptable and we were not resourced to sample biomarkers immediately after exercise, which could have characterised acute, and chronic-on-acute effects of DM-HIIT and usual training. Also, we did not directly assess muscle performance or gather force data, it was not possible to measure impact intensity. Furthermore, intensity was inferred from accelerations, however vertical accelerations could represent vertical trunk displacement during movement without take off and landing, and therefore may not be exclusively a proxy indicator of impact.

The duration of the study was at the lower limit to detect bone change, which could be addressed in future by extending the training period and, as QUS has higher precision error and lower accuracy than DXA, if possible, DXA would be a preferred method to measure bone. This would enable hip and femoral regions to be examined, which have been shown to adapt to high-impact loading in young athlete populations.

Finally, as resources, and acceptability, did not enable biomarker measurement after detraining in HIIT, this effect was not fully characterised.

8.4.2 Conclusions

The present study is unusual in providing longitudinal and comprehensive assessment of biomarkers in a difficult to access performance population, and therefore will add to existing evidence describing gonadal hormone and bone biomarker in dancers. Drawing on results, number of recommendations can be made:

- Dancers are at risk of seasonal reduction in serum 25(OH)D, therefore supplementation, based on blood test to assess vitamin D status, could protect these indoor athletes from impairment associated with vitamin D insufficiency and deficiency.
- Female ballet students should be encouraged to maintain optimal energy balance, to avert menstrual dysfunction and impairment to bone, arising from hypooestrogenism and derangement in LH:FSH coupling
- Even brief (12.5 min) bouts of diverse exercise may be sufficient to reduce permissive bone resorption and therefore this may be an acceptable adjunct in regimens targetting bone health in dancers

8.4.3 Implication for future research

Further studies using the diverse HIIT format could examine the effect of prescribing more frequent training on bone, and extend the duration of training overall (> 24 weeks). Bone responses should be characterised with pQCT, or if feasible, with DXA, to enable visualisation of responses to sites loaded during diverse HIIT, particularly the heel, tibia, and femur.

As there was indication from acceleration profile that capacity to perform repeated high effort actions increased, particularly vertically, this could be formally examined by measuring aerobic capacity, in order to robustly characterise training effect on cardiometabolic fitness.

Finally, using time-motion analysis to supplement accelerometry, and a method to capture ground reaction force, for example shoe-worn sensors,

would enable actions performed during diverse HIIT to be time coded to acceleration profile and integrated with force data. The aim would be to inform future protocol design, by quantifying movements associated with higher intensity dimensions of the acceleration signal and larger magnitudes of ground reaction force, and thus enhance the potential for osteogenic, and aerobic-anaerobic fitness adaptation to diverse HIIT in the future.

9 General discussion

9.1 Summary of Experimental Findings

Studies described in this thesis aimed to address a primary research question: is there an effect on bone of brief intense exercise characterised by diverse movement? The first study, a 16 week RCT in sedentary premenopausal females, compared a team sport intervention with whole-body vibration (WBV), and found an increase in total hip BMD and lean mass in participants assigned to small-sided football (SSG), but no other intervention group, accompanied by acute elevations in biomarkers of bone formation after exercise. Although twice-weekly exercise sessions were brief (15 minutes), attrition was high, and in anecdotal feedback participants who withdrew from the study cited lack of time, and family and work demands, as factors affecting training attendance, particularly for SSG but also WBV. In response, and with the aim of examining dose-response effects by deliberate and specific prescription of physical activity, a novel protocol was developed to replicate, at high density, the diverse, non-uniform actions performed in team sport. Delivered in an audio-visual format, exercise was prescribed in a constrained floor space to increase change of direction (COD) frequency. A feasibility study conducted in pre-, peri- and post-menopausal women found compliance and acceptability for this approach to be high. A further iteration of the protocol was designed and acute physiological responses to a single bout of training examined in sedentary premenopausal females, and female ballet dancers entering their final year of pre-professional training. Analysis of locomotor profile revealed that accelerations were non-monotonic and diverse, with durations for vertical acceleratory and deceleratory actions significantly greater than for other directions during exercise. Furthermore, participants' mean heart rate (HR) during exercise was above 80% of age predicted maximum, exceeding an 80% HR_{MAX} threshold proposed for HIIT, and capillary blood lactate (BLa) concentration immediately after exercise was higher than whole-blood lactate for SSG in Study 1. These findings were encouraging. From a bone perspective, evidence of greater vertical accelerations suggested landing impacts were frequent and it was hypothesised these could provide a stimulus to bone anabolism in a chronic training scenario, if thresholds achieved

exceeded those prevailing during habitual skeletal loading. Furthermore, as exercise conditions imposed high-intensity physiological demands, as demonstrated by elevated exercise HR and BLa, it was hypothesised that the protocol could induce cardiometabolic training adaptations, as seen for regular team sport participation, with chronic practice. Therefore, to test these hypotheses, two longer-term training interventions were conducted, with the aim of examining the effects of chronic administration of diverse movement HIIT (DM-HIIT) on calcaneal bone and indicators of bone metabolism, and on cardiometabolic indicators of exercise intensity.

In a 12 week RCT in sedentary premenopausal females, no effect of three bouts per week of home exercise was observed on bone formation and resorption, characterised by fasted biomarkers at rest, and calcaneal bone ultrasound parameters. However, retested under controlled conditions after 12 weeks training, durations of vertical accelerations at all thresholds increased, and exercise intensity (mean HR% / HR_{MAX} predicted by age) was elevated, suggesting a training effect on acceleratory capacity and greater tolerance of high-intensity conditions for chronic DM-HIIT as home training.

In the final study, female and male ballet dancers trained in small groups, twice weekly and under controlled supervision, with a ramped DM-HIIT programme, which included more challenging choreography than the home exercise study. After 12 weeks, durations of accelerations at all thresholds, and specifically vertically, increased in males and females, yet mean exercise heart rate significantly decreased, indicating a training effect on cardiometabolic capacity, despite ramping of exercise content after 4 and 8 weeks. Whilst no effect on calcaneal bone was observed, bone resorption (characterised by serum CTX-1) was significantly reduced in females assigned to DM-HIIT after 12 weeks, and hip and waist circumference were reduced in dancers who received DM-HIIT, compared with males and females in the year below, who did not supplement classical dance training with diverse HIIT. Alongside bone turnover sampling, this study also examined gonadal hormones and vitamin D (serum 25(OH)D) and as a result, has provided unique insight into biomarker status in a hard-to-access elite athlete population. In a sub-group of females who received DM-HIIT, ~1:1 ratio of luteinising hormone to follicle stimulating hormone (LH:FSH ratio) was significantly elevated, as seen in

hypooestrogenism and polycystic ovarian syndrome (PCOS), and was indicative of significant perturbation along the hypothalamic-pituitary-gonadal axis within this group. Results also showed serum 25(OH)D was significantly reduced at the end of the study in December, compared with basal levels in September, providing compelling evidence of a seasonal effect on vitamin D in this cohort of dance athletes. Taken together, these findings indicate an environment of potential attenuation for anabolic stimuli provided by dance and allied exercise, as a result of menstrual dysfunction in a sub-group of females, and inadequate vitamin D in the entire cohort.

The main findings of studies summarised above are presented in the following tables (Table 39, Table 40), which exclude data from the feasibility study described in Chapter 5. Therefore, Study 2 in the tables, and associated legends for Study 2 participant groups, refers to examination of acute physiological demands of training with the grid, as described in Chapter 6.

Table 39 Effects of Diverse Exercise on Sedentary Females and Male and Female Ballet Dancers

ID	Study Design & Population	Age (y)	Characteristics Duration (w), frequency (per week), session duration (min), supervised (S) non-supervised (NS)	Bone Outcomes DXA: Study 1, BMD, BMC QUS: Study 2-4, SI, BUA, SOS	Bone Metabolism CTX-1: resorption; P1NP: formation; OC: formation UT: Acute Response	 T: Acute Response	 Detraining Response	Biomarkers Study 2 CRP Study 4 GnH: E, FSH, LH (F); T, SHBG (M); $25(OH)D_S$
1	RCT Training Study Sedentary pre-menopausal F	SSG 29-46 WBV 30-44 CON 34-46	16; 2; 13.5; S 16; 2; 13.5; S Habitual PA	↑Tot Hip* 0.010 kg ↔ BMD, BMC, WB & ROI ↔ BMD, BMC, WB & ROI	↑P1NP* ↑OC* ↔CTX-1 ↔P1NP ↑OC*↔CTX-1 -- -- -- -- -- -- -- -- -- --	↑P1NP* ↔OC ↔CTX-1 ↔P1NP ↔OC ↔CTX-1 -- -- -- -- -- -- -- -- -- --	-- --	-- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- --
2	Acute (Single Bout) characterisation Sedentary pre-menopausal F Ballet dancers F	SF 27-45 DF 17-22	-- -- 14.5; S -- -- 14.5; S	L, R Absolute SF vs DF: SI: ns; BUA: ns; SOS: ns BUA_{BMNORM} SF vs DF: R BUA**: DF 0.44 AU > SF L BUA**: DF 0.33 AU > SF	-- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- -- -- --
3	RCT Training Study Sedentary F	DVD 24-49 CON 23-49	12; 3; 14.5; NS Habitual PA	L,R Absolute DVD vs CON: SI: ns; BUA: ns; SOS: ns BUA_{BMNORM} DVD vs CON: R BUA: ns; L BUA: ns	12 vs 0w:↑11% P1NP ns 12 vs 0w: ↔ P1NP	12w vs 0w: ↔ CTX-1 12w vs 0w: ↔ CTX-1	-- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- -- --	↔ CRP ↔ CRP
4	Prospective Non-randomised Intervention Pre-professional ballet dancers M, F	$DMHIIT_F$ 17-22 TAU_F 17-19 $DMHIIT_M$ 17-20 TAU_M 17-19	12; 2; 12 (R1-2), 11.5 (R3) S Usual dance training 12; 2; 12 (R1-2), 11.5 (R3) S Usual dance training	$DMHIIT_F$ vs TAU_F : L, R SI: ns; BUA: ns; SOS: ns $DMHIIT_M$ vs TAU_M : L SI: ↑16% $DMHIIT_M$ vs ↓2% TAU_M (p = 0.055) ↑L SOS*, ↑R SOS* (*main effect of time in males*)	12 vs 0w: ↔ P1NP 12 vs 0w: ↔ P1NP 12 vs 0w: ↔ P1NP 12 vs 0w: ↔ P1NP	12 vs 0w: ↓CTX-1* 12 vs 0w: ↔CTX-1 12 vs 0w: ↓CTX-1* 12 vs 0w: ↓CTX-1* (*main effect of time in males*)	↔P1NP ↑13% CTX-1*** -- -- -- -- -- -- -- -- -- -- ↓12%P1NP* ↑22% CTX-1* -- -- -- -- -- -- -- -- -- --	↔ GnHs ↔ GnHs ↔T ↑SHBG* ↔T ↑SHBG* (*main effect of time in males*) ↓25(OH)D*** F, M (*main effect of time*)

DXA: Dual X-ray Absorptiometry; BMD: Bone Mineral Density; BMC: Bone Mineral Content; QUS: Quantitative Ultrasound Scan (Calcaneal); SI: Stiffness Index; BUA: Broadband Ultrasound Attenuation; SOS: Speed of Sound; CTX-1: C-terminal telopeptide of Type 1 Collagen; P1NP: N-terminal Propeptide of Type 1 Procollagen; OC: Osteocalcin; CRP: C-reactive Protein; GnH: Gonadal Hormone; E: Estradiol; FSH: Follicle Stimulating Hormone; LH: Luteinising Hormone; F: Female; T: Testosterone; SHBG: Sex Hormone Binding Globulin; M: Male; 25(OH)DS: Serum 25-Hydroxyvitamin D; UT: Untrained; T: Trained; RCT: Randomised Control Trial; SSG: Small-Sided Football Games; WBV: Whole-Body Vibration; CON: Controls; PA: Physical Activity; WB: Whole-Body; ROI: Regions of Interest; SF: Sedentary Females; DF: Female Dancers; ns: non-significant; BUA_{BMNORM}: BUA Normalised to Body Mass; AU: Arbitrary Units; DVD: Home exercise; DMHIIT: Diverse Movement HIIT; R: Ramp.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 40 Physiological Demands of Diverse Exercise in Sedentary Females and Male and Female Ballet Dancers

ID	Study Design & Population	Int Group	Exercise Heart Rate Study 1-4: Mean HR (bpm); Peak HR (bpm); (Mean HR/ HR_{AGEMAX})%		Acceleration Profile, Lactate, Perception of Effort Duration (s) > 2 g Up; Lactate# ($mmol.L^{-1}$); $VAS_{CHALLENGE}$¥ (AU)		Body Composition Study 1:Δ↑↓TLM (kg), % Basal FM_{AN} Study 2:↑↓BM (kg), ↑↓BMI ($kg.m^2$) $Mean_{⌂}$ Study 3: %↑↓ Basal W:H (AU) Study 4:Δ↑↓WC, HC (cm) DXA: Study 1; MRI: Study 3
			UT: Acute Response	T: Acute Response	UT: Acute Response	T: Acute Response	
1	RCT Training Study Sedentary pre-menopausal F	SSG	157±12; 180±6; 87±8	149±10 ns ↓174±7* 74±23% ns	-- -- -- 3.2±1.4* 59±2 ↕ sig	-- -- -- ↓2.1±1.3 ↓54±1 ns ↕ ns	↑0.770 kg TLM* 7±14% FM_{AN}‡
		WBV	↔ HR	↔ HR	-- -- -- 0.8±0.4 -- -- --	-- -- -- ↑1.4±0.5 -- -- --	↑0.060 kg TLM ns 1±6% FM_{AN} ns
		CON	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	↑0.209 kg TLM ns 0±10% FM_{AN} ns
2	Acute (Single Bout) characterisation Sedentary pre-menopausal F Ballet dancers F	SF	158±11; 187±14; 86±5 ↕ ns ↕ ns ↕ns	-- -- -- -- -- -- -- -- -- --	3.9±1.7 7.6±4.1 65±20 ↕ ns ↕ ns ↕ ns	-- -- -- -- -- -- -- -- -- --	53.1±5.6 kg*** 19.1±1.5 $kg.m^2$ *** ↕ sig ↕ sig
		DF	166±17; 193±12; 83±9	-- -- -- -- -- -- -- -- -- --	3.4±2.8 8.3±1.0 58±15	-- -- -- -- -- -- -- -- -- --	67.9±2.4 kg 24.4±4.1 $kg.m^2$
3	RCT Training Study Sedentary F	DVD	142±16; 171±15; 79±9	↑150±11 ns ↑174±9 ns ↑83±5 ns	2.4±1.1 -- -- -- 6±1¥	↑4.9±4.5≈ -- -- -- ↑8±2¥ ns	-0.8% W:H ↔SCT_{AR} & $_{VOL}$ ↔$VISC_{AR}$ & $_{VOL}$ ↕ ns⁶ ↕ sig‼ ↕ sig‼ ↕ ns ↕ ns
		CON	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	3.8% W:H ↔SCT_{AR} & $_{VOL}$ ↔$VISC_{AR}$ & $_{VOL}$
4	Prospective Non-randomised Intervention Pre-professional ballet dancers M, F	$DMHIIT_F$	166±17; 193±12; 83±9	↓160±13* ↓190±9 ns ↓79±6*	3.4±2.8 8.0±4.4 58±15	↑7.9±4.9**** ↓6.5±2.5 ns ↓43±17**	↓3.3±3.7** WC ↓3.4±3.7* HC
		TAU_F	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	↓0.6±1.8 ns WC ↓0.4±3.1 ns HC
		$DMHIIT_M$	177±11; 201±12; 88±5	↓164±11* ↓192±10* ↓81±5*	4.2±2.4 8.8±2.8 55±21	↑7.4±3.2* ↓8.3±2.8 ns ↓51±13 ns	↓3.6±2.3** WC ↓4.3±3.0** HC
		TAU_M	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	-- -- -- -- -- -- -- -- -- --	↓2.2±2.7 ns WC ↓0.7±2.1 ns HC

Int Group: Intervention Group; Bpm: Beats per minute; HR: Heart Rate; HR_{AGEMAX}: Maximum Heart Rate (220-age[y]); Lactate# : Whole-Blood (Study 1), Venous Capillary Sample (Study 2, 4); $VAS_{CHALLENGE}$¥ : Visual Analogue Scale rating of Exercise Challenge (Study 1, 2, 4), ¥ RPE 1-10 (Study 3); AU: Arbitrary Units; DXA: Dual X-Ray Absorptiometry; MRI: Magnetic Resonance Imaging; Mean⌂: Estimate of mean from Two-way Mixed Anova used to represent between-group comparison (Study 2); RCT: Randomised Control Trial; SSG: Small-Sided Football Games; WBV: Whole-Body Vibration; CON: Controls; ns: non-significant; sig: significant; TLM: Total Lean Mass; FM_{AN} :Android Fat Mass; SF: Sedentary Females; DF: Female Dancers; DVD: Home exercise; W:H : Waist to Hip ratio; SCT_{AR} &SCT_{VOL} : Sub-cutaneous Areal & Volumetric Adipose Tissue; $VISC_{AR}$ & $_{VOL}$: Visceral Areal & Volumetric Adipose Tissue; $DMHIIT_{F/M}$: Diverse HIIT Female/Male dancers; $TAU_{F/M}$:Training As Usual Female/Male dancers; WC: Waist Circumference (cm); HC: Hip Circumference (cm).
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ≈ $p = 0.062$; ‡ $p = 0.081$; ⁶ $p = 0.059$; ‼ main effect of group ($p < 0.05$).
↑ ↓ Indicator of direction of change in units of parameter for mean post-intervention versus mean basal.
↕ Indicates comparison between interventional groups for parameter.
↔Indicates no change within group for parameter.

9.2 Comparison of Diverse Exercise Effects Across Studies

9.2.1 Physiological demands of acute exposure

Using indicators of exercise intensity to directly compare data from Studies 1 - 4 provided further insight into cardiometabolic demands of diverse exercise, which according to results appeared to depend on context of delivery. Exercise intensity, characterised by mean exercise heart rate (HR) as a percentage of age-predicted maximum (220- age [y]), tended to be significantly different between intervention groups during a single bout of training (p = 0.060, η^2 = 0.138), and was lowest (79 ±9%) in premenopausal sedentary females during baseline training in Study 3, where diverse exercise was applied in the grid format at reduced complexity. Exercising according to this format, but at higher density of prescribed actions, training intensity in female ballet dancers was 83 ±9% and in sedentary females reached 86 ±5%, replicating 86 ±8% for basal training intensity during small-sided football (SSG) in a sedentary female population in Study 1. At 88 ±6%, highest intensity for a single bout of diverse exercise was observed in male ballet dancers during diverse-movement HIIT (DM-HIIT) at baseline testing in Study 4 (Figure 36).

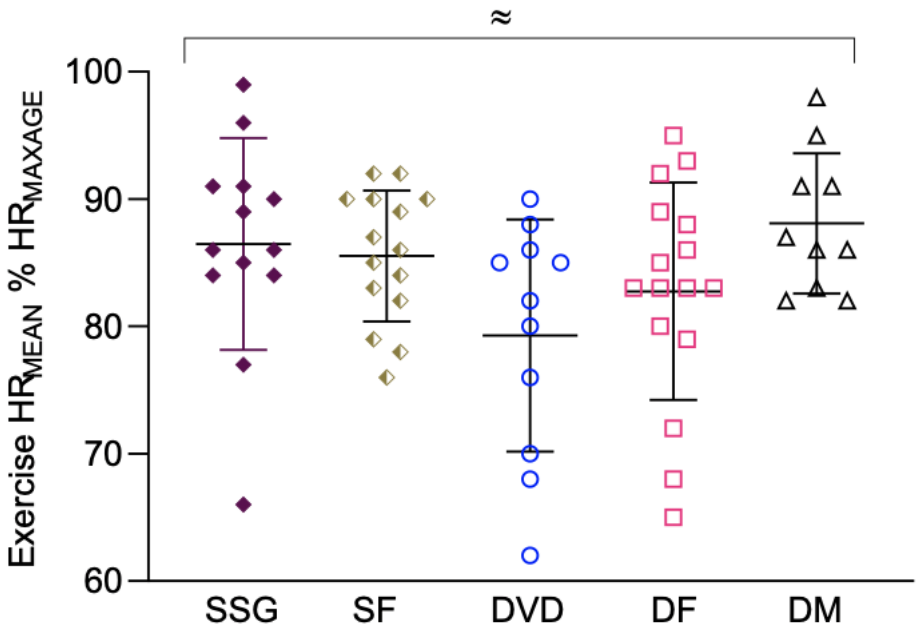


Figure 36 Training Intensity During an Initial Bout of Diverse Exercise

SSG: Small Sided Games; SF: Sedentary Females; DVD: Home Exercise; DF: Female Dancers; MF: Male Dancers. ≈ p = 0.060.

It may therefore be concluded that in untrained females diverse exercise applied as SSG and as DM-HIIT achieved, or closely approximated, a threshold of 80% HR_{MAX} proposed for HIIT (Buchheit & Laursen, 2013), and in male dancers was 2 % lower than 90 – 95%, the intensity at which performance benefits have been demonstrated in athletes participating in intermittent sports codes (Engel, Ackermann, Chtourou, & Sperlich, 2018). An additional finding from comparison of indicators of exercise intensity was that blood lactate (BLa) concentration, after a single bout of training in the basal state, was significantly higher after diverse exercise prescribed as DM-HIIT than as SSG ($p < 0.001$, $\eta^2 = 0.508$) (Figure 37), yet despite the disparity in BLa responses, perception of exercise challenge in the basal state did not differ significantly between interventional groups across studies ($p = 0.693$, $\eta^2 = 0.037$) (Figure 37).

Despite evidence of greater physiological demand for acute grid training compared with football, metabolic response cannot be assumed to be an indicator of enhanced osteogenic potential for diverse HIIT. As the determinants of anabolic effects of exercise on femoral geometry and BMD appear to be related to mechanical dimensions of the loading signal, greater elevation in BLa after grid exercise cannot be presumed to indicate a higher osteogenic index per se. For example, elite swimmers train at high cardiometabolic intensities and yet demonstrate lower regional and whole-body BMD than their non-acquatic athlete counterparts (Bellver et al., 2019), which is attributable to the different training environments and mechanical loading conditions in which they habitually perform (Warner, Shea, Miller, & Shaw, 2006).

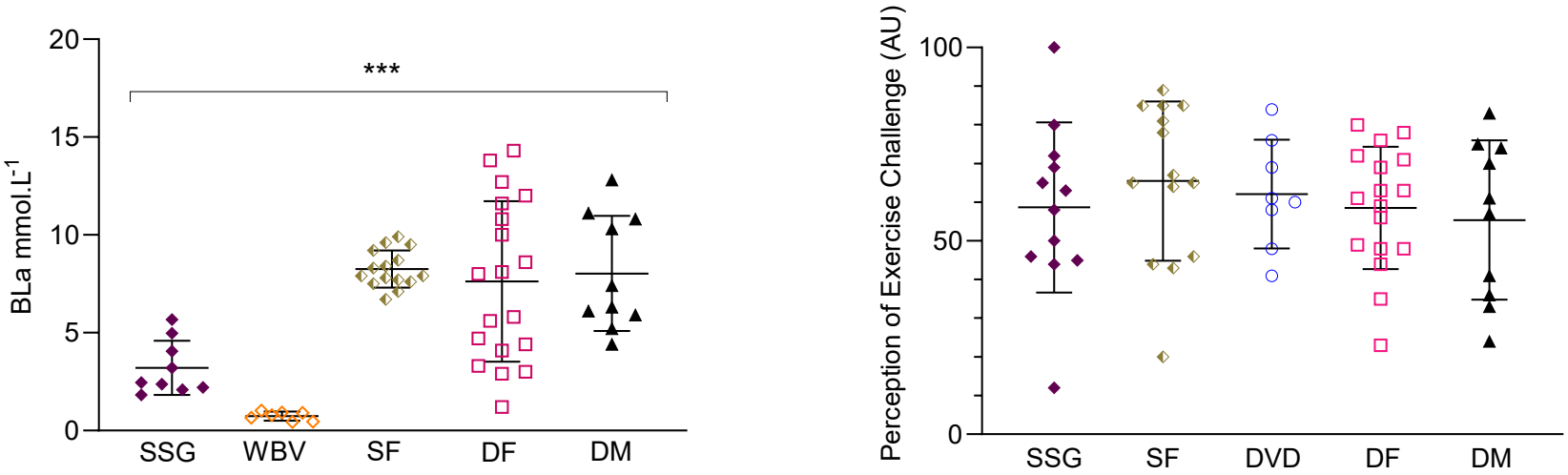


Figure 37 Blood Lactate and Perception of Exercise Challenge Studies 1 – 4

SSG: Small-Sided Games; WBV: Whole-Body Vibration; SF: Sedentary Females; DVD: Home Exercise; DF: Female Dancers; DM: Male Dancers.
Note[a]. Lactate: whole-blood (Study 1, SSFT vs WBV); SF, DF, DM: Venous capillary blood (Study 2, Sed Females vs Dancers; & Study 4 12w DM-HIIT in dancers).
Note[b]. Perception of effort: VAS (Studies 1, 2, 4); RPE (1 – 10*100): (Study 3, Home Exercise).
*** $p < 0.001$.

This finding is somewhat surprising, given that alignment between cognitively mediated perception of exercise effort and BLa, as indicators of physiological stress and relative training demand, forms the basis of recommendations for their use to monitor exercise intensity (Norton, Norton, & Sadgrove, 2010). It is possible that despite significantly higher lactate observed in participants after a single bout of DM-HIIT than SSG differences in basal training status, and accommodation to usual activity demands, affected subjective rating of exercise effort. For example, dancers may not have perceived the effects of relatively elevated lactate as unusual; ballet dancers are habituated to 6 – 8 hours of daily practice, during which male dancers have been shown to achieve 95% and females 85% of maximal heart rate in response to brief, high-intensity actions during class (Cohen, Segal, Witriol, & McArdle, 1982), and after intense phases of performance blood lactate of ~10 $mmol.L^{-1}$ in dancers has been reported (Schantz & Åstrand, 1984). However, untrained sedentary females who trained with the same diverse protocol applied in male and female dancers' did not differ from the dancers in their rating of exercise challenge ($p > 0.999$, all pairwise comparisons), which does not support speculation that dissociation between results for blood lactate and perception of effort could be partly attributable to differences in background training status. It may even be hypothesised that traversing short (3 m maximum) distances in a constrained exercise space created a perceptual illusion, akin to that of the 'wine glass' effect, whereby properties in one domain ('*heavier glass'*) are shown to influence perceptual rating in another (*'higher quality wine'*) (Hummel, Delwiche, Schmidt, & Hüttenbrink, 2003). Accordingly, it could be hypothesised that lack of progression ('*not going anywhere!'*) during exercise may have given rise to a perception of lower exercise demand ('*not doing very much!*'), by a process of attributional transference. Certainly, given that training intensity for SSG was matched by diverse HIIT in females but elicited a significantly lower BLa response, results for initial exposure in this population suggest that rating of exercise challenge was more closely aligned to relative intensity characterised by training HR as $\%HR_{MAX}$ than by BLa. This observation is in agreement with what has been previously found for HIIT, whereby high (> 85% HR_{MAX}) HR during training does not necessarily result in high BLa (Buchheit & Laursen, 2013; Tschakert et al., 2015).

9.2.2 Comparison of locomotor profile for single bout diverse HIIT

Across studies, BLa responses demonstrated that a single bout of DM-HIIT highly taxed the glycolytic pathways for energy production, and therefore accelerometry profile during initial training was also compared, to examine the relationship between locomotor characteristics and acute physiological responses. As the motion sensor unit used in sedentary females in Study 1 was of an early iteration (GPSport, Canberra, Australia) and did not describe accelerations according to orthogonal, or thresholds above 1.5 g, data from this study were excluded, as were data from females in Study 3, who trained with a diverse HIIT protocol modified to provide less intense actions. Therefore only data from sedentary females in Study 2, and female and male dancers in Study 4, who exercised with the same regimen of diverse HIIT constrained to 3 x 3 m training area (grid format) were used, to compare locomotor profile in the untrained state, according to duration of vertical accelerations (s) at three thresholds (above 1 g, 2 g and 3 g). Analysis showed the groups exhibited significantly different vertical acceleration characteristics ($p = 0.003$, $\eta^2 = 0.181$, group*threshold [>1 g,>2 g, >3g]): in sedentary females duration of vertical accelerations above 1 g was 20.2 ±7.3 s less than in male dancers ($p = 0.026$) and also 9.9 ±6.0 s less than in female dancers ($p = 0.321$) however, above 2 g and 3 g, vertical acceleration characteristics did not differ between groups ($p < 0.001$, $\eta^2 = 0.924$, main effect of threshold) (Figure 38).

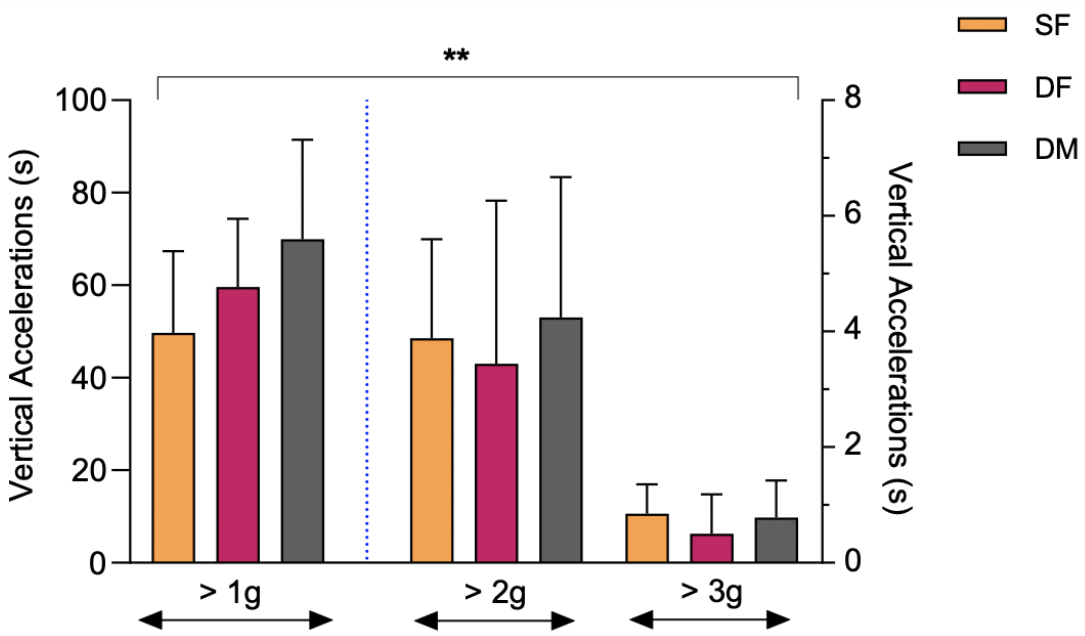


Figure 38 Vertical Accelerations During Diverse Exercise at Baseline[b]

SF: Sedentary Females (Study 2); DF: Female Dancers (Study 2); MF: Male Dancers (Study 4). *Note*[a]. Values for columns to right of vertical blue line on right Y axis. [b] Denotes untrained state. ** $p < 0.01$.

Whilst this finding is not unexpected, as significantly greater vertical accelerations at the lowest threshold (1 – 1.5 g) were observed for diverse HIIT in all interventional groups (Studies 2 – 4), it further supports the hypothesis that acute elevation in blood lactate observed immediately after a single training bout was predominantly attributable to actions requiring vertical accelerations and decelerations, as these occurred most frequently during diverse exercise in this format. Moreover, male dancers exhibited the highest mean training HR (88 ±6% HR_{MAX}) and longest duration of vertical accelerations, lending further evidential support to the proposal that vertically oriented actions, such as jumps, lateral cutting and landings, imposed the greatest cardiometabolic demand during diverse HIIT in the basal state.

Lack of observed difference in high threshold vertical accelerations between experimental groups is less expected and difficult to explain. Unlike sedentary women, during daily class and rehearsals male and female ballet dancers perform high intensity jumping and floor traversing activities, which require brief bursts of all-out effort (Koutedakis & Jamurtas, 2004), and yet this difference in basal state activity profile did not result in longer durations for accelerations above 2 g and 3 g in dancers. It is possible that the constrained

exercise space prevented dancers moving at speeds they would achieve during habitual ballet jumps and progressive cross-floor sequences, and therefore the effect of performing diverse actions within the grid obliterated differences in locomotor profile, which might have been observed in a more expansive exercise area. It may also be speculated that lack of familiarity with activities prescribed, which replicated team sports rather than classical ballet steps, attenuated higher intensity efforts in a similar manner across interventional groups, however, results for BLa and HR intensity indicate this was not a barrier to exercising under high intensity conditions. This is an important finding to consider in future implementation of diverse HIIT, as it suggests that specialism in one exercise domain did not confer an advantage in a one-off training bout, and vertical acceleration and deceleration demands highly taxed anaerobic metabolism in both dancers and sedentary females. In cross-sectional examination of cardiometabolic fitness using an incremental treadmill test, dancers were found to have lower VO_{2MAX} than in swimmers, gymnasts, endurance runners and volley-ball players (Angioi et al., 2009), and physiological profiling of dance styles has highlighted inadequacies in classical dance training for preparing dancers for the high anaerobic demands of classical performance (Wyon et al., 2011). Therefore, it is possible that anaerobic metabolism had not been selectively targeted in usual ballet training in these cohorts of young dancers, which could explain the observation that dancers did not outperform sedentary participants in high-threshold vertical accelerations, as they were not adapted to repeated high intensity efforts under conditions of sustained anaerobic demands. It would be interesting to explore this speculation by exposing athletes from other performance codes to a single bout of DM-HIIT, in order to test the hypothesis that higher threshold accelerations during diverse exercise in this format are an indicator of anaerobic capacity. The alternative hypothesis, that high threshold accelerations are attenuated during diverse HIIT by constraining performance area, could be examined in future by manipulating dimensions of the exercise space and characterising the effect on acceleration profile.

9.2.3 Comparison of bone measures in the untrained state

In Study 1, serial measurement of plasma markers of bone turnover (BTM) before and after exercise was undertaken however, in subsequent

studies, a serum BTM sample was obtained at rest in the fasted state, as resources either did not extend to cover assay costs for serial assessment (Study 3), or cannulation was unacceptable (Studies 2 and 4). Where available, samples of bone formation and bone resorption were compared to give a cross-sectional indication of bone metabolic status on entry to each study.

Fasted P1NP, a marker of bone formation, and CTX-1, a marker of bone resorption, were elevated in dancers compared with sedentary females ($p < 0.001$) (Figure 39 a, b), which was attributed to the significant difference in age and maturation status between experimental groups.

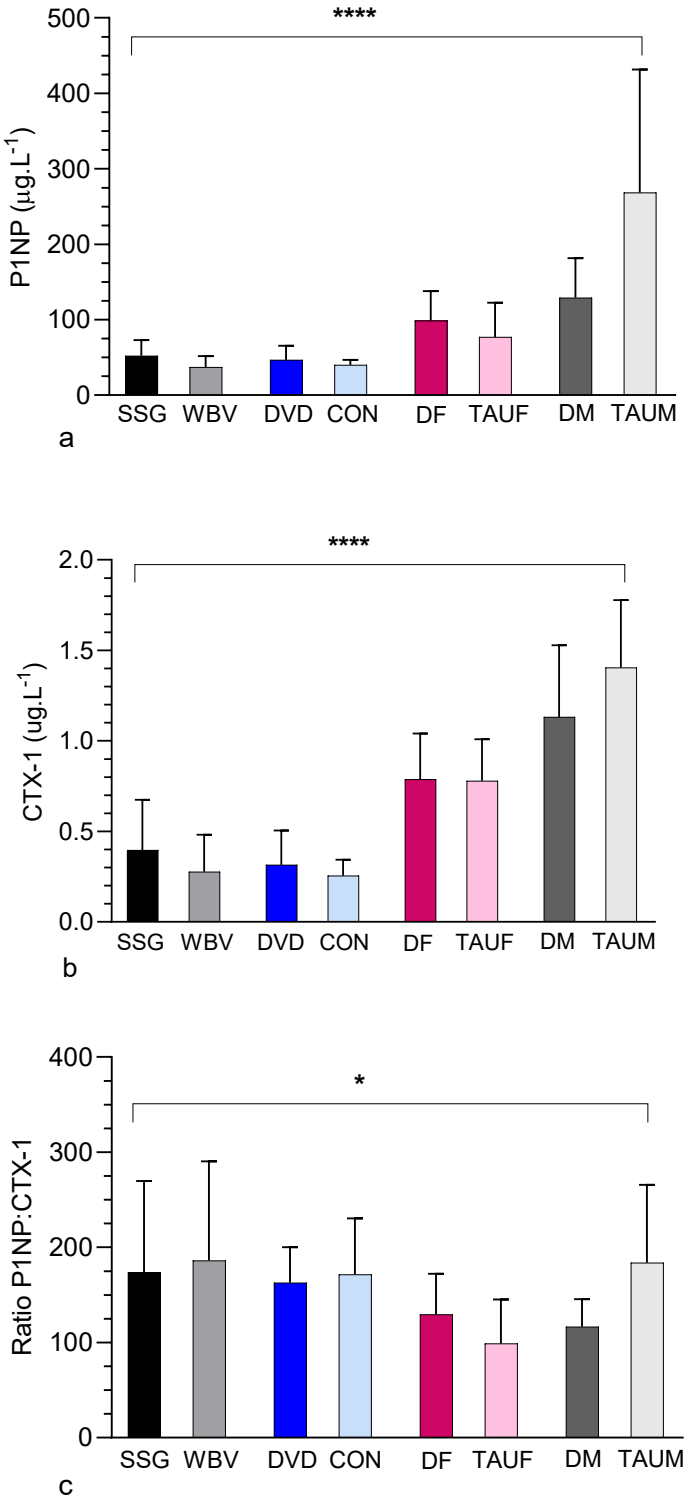


Figure 39 (*a-c*) Comparison of Biomarkers of Bone Turnover in the Basal State

P1NP: N-Terminal Propeptide of type-1 Procollagen; CTX-1: C-Terminal Telopeptide of Type-1 Collagen; SSG: Small Sided Games; WBV: Whole-Body Vibration; DVD: Home Exercise; CON: Control; DF: Female Dancers assigned to DM-HIIT; TAUF: Female Dancers assigned to Training As Usual; DM: Male Dancers assigned to DM-HIIT; TAUM: Male Dancers assigned to TAU. **** $p < 0.0001$; * $p < 0.05$.
Note. SSG, WBV: plasma sample; DVD, CON, DF, TAUF, DM, TAUM: serum sample.

Bone turnover markers are observed to rise as the pubertal growth spurt is initiated in response to trophic hormones (Rauchenzauner et al., 2007), therefore higher BTMs in dancers is in accordance with the increase in bone biomarkers observed at puberty and sustained into late adolescence (Jürimäe, 2010). Whilst results require cautious interpretation for several reasons (low numbers in participant groups, difference between analyte medium for BTM assay, inability to distinguish bone modelling from bone remodelling), pairwise comparisons revealed a hierarchy for dynamic bone metabolism characterised by BTMs, which was higher in young dancers than sedentary females, in male dancers than female dancers, and significantly higher in second year males than all other experimental groups. Results are in agreement with evidence of high and prolonged elevation in bone turnover markers during, and in the first years after, the pubertal growth phase in males (Szulc, Kaufman, & Delmas, 2007), with lower values reported in females, in whom bone biomarkers decline steadily from peak values at the end of puberty (Van Coeverden et al., 2002). However, as formal examination of pubertal status was not undertaken in dancers and access to DXA, which would have supplied site-specific bone measurement, was not available, it can only be stated with confidence that results in dancers offers global confirmation of what has previously been shown for maturation-related elevation in BTMs in pubertal adolescents (Rauchenzauner et al., 2007; Zürcher et al., 2020).

Evaluation of basal BTMs did yield insight into a potential strategy for implementing positive effects on bone in female dancers: whereas P1NP did not differ in any pairwise comparison between dancers and sedentary females, CTX-1 was significantly higher in young ballet dancers, and consequently ratio of formation to resorption was lower in these cohorts than in sedentary females (Figure 39 c), although in post hoc comparison this was not found to be significant. This could be attributable to greater bone modelling in dancers associated with relatively recent (or active) skeletal growth, however it could also signify the presence of relative energy deficiency (in) sport (REDS), given evidence of increased bone resorption in states of REDS in exercising females (De Souza et al., 2008), and of higher risk of subtle and profound endocrine disturbance in athletes in aesthetic disciplines, such as classical ballet, as a result of REDS associated dysfunction along the hypothalamic-pituitary-gonadal

axis (Elliott-Sale et al., 2018). In cross-sectional examination of calcaneal bone at baseline (Figure 40 a-b) per unit body mass, female dancers exhibited higher calcaneal bone stiffness than sedentary females, on account of dancers' lower body mass (Figure 40c), yet no difference between female cohorts was found for absolute calcaneal BUA, which were adequate against reference values.

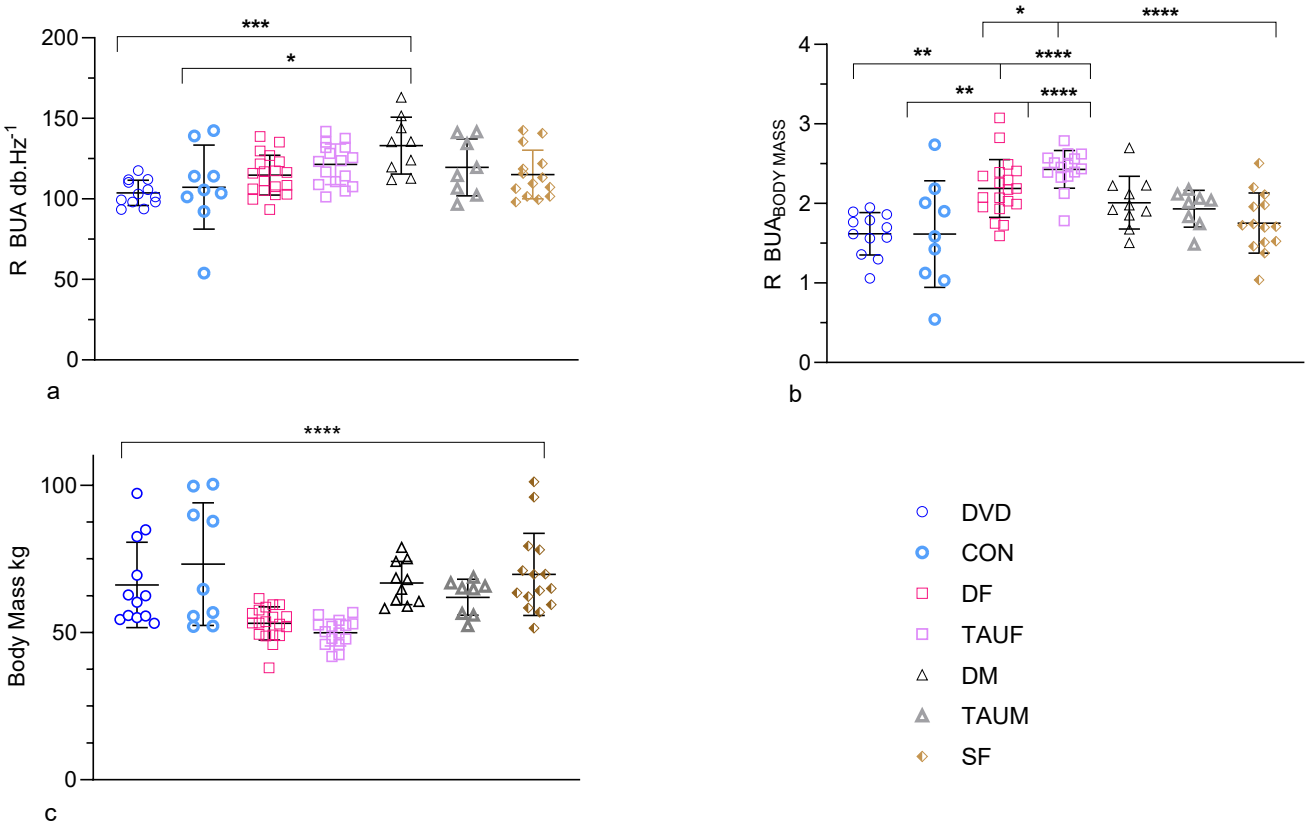


Figure 40 Right Calcaneal BUA *(a, b)* and Body Mass (*c*) in Diverse Exercise Intervention Groups

BUA: Broadband Ultrasound Attenuation; DVD: Home Exercise; CON: Control; DF: Female Dancers assigned to DM-HIIT; TAUF: Female Dancers assigned to Training As Usual; DM: Male Dancers assigned to DM-HIIT; TAUM: Male Dancers assigned to TAU.
**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

As a surrogate indicator of long-term loading in calcaneal bone, higher BUA normalised to weight strongly suggests bone anabolic effects of regular dance training, and this conclusion is in agreement with meta-analytic findings of greater BMD in young female dancers, compared with age-matched non-dancers, localised to the hip and trochanteric region, which are highly loaded during dance performance (Wewege & Ward, 2018). Cross-sectional evidence has described potential dose-response effects for physical activity in eliciting bone anabolic effects in young and adolescent females (Kambas et al., 2017) and dancers' training, which begins early in childhood and entails hours of daily practice, certainly aligns with the emergent message of 'exercise early and often' to promote lifelong bone health (Troy et al., 2018). Nevertheless, as bone resorption in females, but not in males, has been demonstrated to upregulate rapidly in response to short-term (5 days) energy restriction (15 kcal.kg $LBM^{-1}.d^{-1}$) (Papageorgiou et al., 2017), if energy availability were compromised, for example in response to increases in rehearsal and performance demands, or higher drive for thinness in preparation for professional auditions, dancers could experience an acute elevation in bone resorption. Furthermore, elevated LH to FSH ratio was observed in a sub-group of third year females who received diverse HIIT, which could have attenuated bone anabolic effects of exercise in accordance with dysregulation of oestrogen in states of low energy availability in female athletes (De Souza & Williams, 2004; Williams et al., 2019). Therefore, whilst a pro-bone effect of daily PA may be inferred retrospectively from QUS profile, reducing resorption as was demonstrated for chronic diverse HIIT in female dancers, may present a viable approach to favouring net bone anabolism and offset the potential impact of RED associated effects on bone, for which evidence was found in this population.

9.2.4 Chronic effects of diverse exercise compared across studies

Capacity to perform a greater number of actions specific to the format of diverse exercise prescribed, at reduced intensity, was the most consistently supported chronic training effect of studies described in this thesis. In Study 1, a strong trend for sedentary females to run and sprint greater distances after 16 weeks SSG, at a dose of 2 x 13.5 min per week, was accompanied by a 4 ±13% reduction in exercise HR (mean HR % age predicted HR_{MAX}) and 35 ±32% lower BLa immediately after exercise (Figure 41 a-b). In Study 3,

sedentary females undertook 3 x 14.5 min weekly diverse HIIT at home and whilst BLa was not examined, HR intensity, as described for SSG, increased by 5 ±9% after 12 weeks (Figure 41 a) and duration of vertical accelerations increased above all thresholds, with the greatest change, albeit with the widest dispersal, seen for high threshold accelerations above 3 g (Figure 42).

This effect was also seen in male and female dancers who undertook 2 x 12 min supervised diverse HIIT for 12 weeks, with the largest increase in duration of high-threshold acclerations in female dancers (

Figure 42). High-threshold acceleration profile in female dancers was significantly different than in the basal training state, whereas it was not significantly different in males, and the increase was highly direction-specific: duration of vertical accelerations was 1.0 ±02 s longer after 12 weeks DM-HIIT. Expressed in counts (derived from 100 Hz sampling rate) this represents ~100 count increase in high-threshold accelerations, which is in alignment with the volume of high-intensity acceleration reported to be relevant to bone health within daily PA (Stiles et al., 2017). Moreover, as CTX-1 decreased, but only in in female dancers, after 12 weeks' training, indicating a reduction in background bone resorption, it is interesting to speculate whether exposure to unusual high-intensity accelerations provided by the protocol could have elicited the observed effect on bone metabolism. The changes in accelerometry profile, specifically increases in duration of vertical accelerations at all thresholds, were accompanied by a decrease in dancers' exercise HR intensity of 4 ±5% in females and 8 ±7% in males, without any reduction in BLa post-exercise after 12 weeks diverse HIIT, unlike after 16 weeks SSG (Figure 41 a, b).

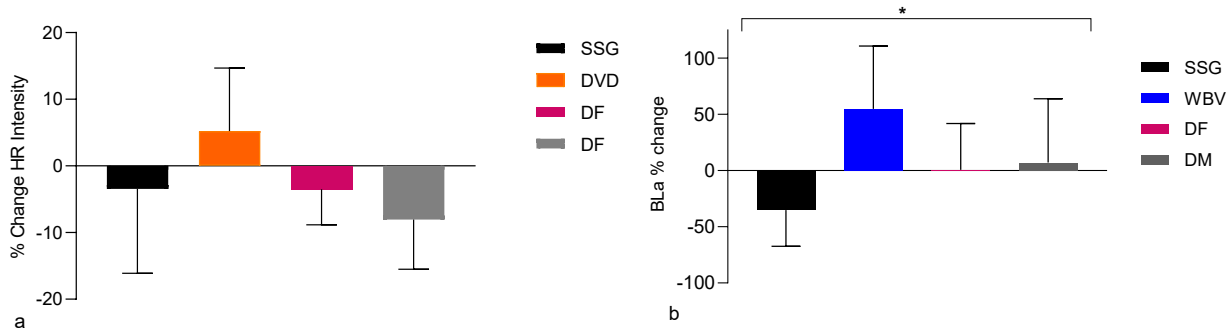


Figure 41 Change in HR Intensity and Blood Lactate Concentration after Chronic Training

SSG: Small Sided Games; DVD: Home Exercise; DF: Female Dancers; MF: Male Dancers; WBV: Whole-body Vibration. * $p < 0.05$.

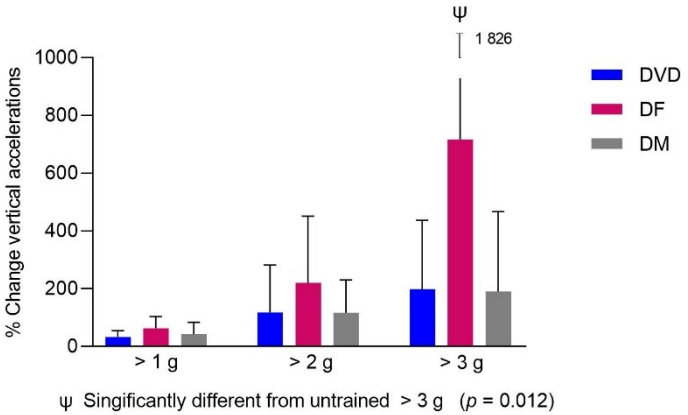


Figure 42 Change in Vertical Acceleration Profile after 12 Weeks Diverse-Movement HIIT

DVD: Home Exercise; DF: Female Dancers; MF: Male Dancers. Ψ Significantly increased above 3 g after 12 weeks DM-HIIT.

A number of conclusions may be drawn from these findings: whilst both diverse HIIT and football as small-sided games (SSG) elicited training adaptations in participant groups examined, whereby high demand actions occurred more frequently at reduced exercise intensity, the burden imposed on anaerobic metabolism was not diminished by chronic training with diverse HIIT, unlike after chronic SSG. This suggests that ramping of the cardiometabolic stimulus elicited by diverse exercise was more successful for HIIT in the grid format compared with a team sport activity played under intensive conditions.

Whilst there is not yet an established taxonomy for HIIT, whereby definitions according to protocol, indicators of intensity and outcome measures are commonly agreed (Campbell et al., 2019), recent research has focussed on work to rest ratios, as an approach to describing parameters and potential benefits of HIIT exercise regimens (Tschakert et al., 2015; Warr-di Piero, Valverde-Esteve, Redondo-Castán, Pablos-Abella, & Sánchez-Alarcos Díaz-Pintado, 2018). In relation to evidence provided by these investigations, BLa in response to diverse exercise applied as SSG (sedentary females, Study 1) aligned with values for short-interval HIIT, characterised by 10 s activity followed by 10s recovery, whereas significantly higher BLa after DM-HIIT was more typical of intermediate-long duration HIIT (Warr-di Piero et al., 2018). Accepting a taxonomy for diverse exercise based on durational characteristics of training and recovery, it follows that amplitude of cardiometabolic responses for acute and chronic exposure may partly be explained by the effect of delivery format on work to rest relationships, and the relative demands imposed on aerobic and glycolytic metabolism as a direct consequence. If work to rest duration and frequency of high-demand actions are temporal drivers of exercise intensity, their accessibility to external control provides a plausible mechanism for ramping physiological responses to training. Evidence in both an athlete (Study 4) and sedentary (Study 3) population that diverse HIIT provided a superior, progressive stimulus to exercise-induced metabolic adaptation than SSG (Study 1) may therefore partly be attributed to capacity to leverage these drivers, which was a priori greater for diverse exercise prescribed as HIIT. In this format exercise actions were pre-determined, participants were familiarised to them and performance intensity was externally imposed, whereas during SSG sedentary females exercised under the open-code and dimensionally less constrained conditions characteristic of team sports (Vaeyens et al., 2008),

gaining skills that may have enabled football specific actions to be performed at lower demand after longer term practice (Hill-Haas et al., 2011).

In support of this proposal, analysis of locomotor profile and GPS during SSG showed that team sport actions performed by untrained female were episodic and brief (~2 – 3 s), as previously demonstrated for small-sided game play in this population (Krustrup, Helge, et al., 2018), and were typically followed by fallow periods of walking or standing, which increased significantly after 16 weeks training. Both acutely and chronically this pattern of activity could explain the finding of significantly lower BLa after SSG than after DM-HIIT, as a result of lactate clearance during recovery phases. In contrast, BLa after DM-HIIT, which aligned with lactate values observed for 90 s work : 90 s recovery designated as intermediate-longer duration HIIT (Warr-di Piero et al., 2018), reflected deliberate prescription of diverse exercise actions in longer (60 s) bouts in an overall train to recovery ratio of 1 : 0.6 for the duration of the protocol.

Overall, results for these studies support the hypothesis that context and format of delivery exert quantifiable effects on physiological demand, and this finding could be exploited in future investigation, depending on the cardinal aim of exercise prescription. For example, results showed that using a constrained floor grid to implement diverse HIIT imposed high demands on anaerobic metabolism, which could benefit athletes engaged in intermittent sports and aesthetic performance codes such as ballet, for which there is preliminary evidence that greater cardiorespiratory fitness is associated with superior technical and aesthetic competence (Angioi et al., 2009). Anecdotally, expert feedback from ballet and dance technique teachers, after Study 4 was completed, confirmed this relationship. In unsolicited and informal rating of effects of the protocol teachers reported that they would retain DM-HIIT, as it was perceived to have increased dancers' leanness, improved *'stamina*' (term used by ballet staff in preference to 'fitness') during class, and consequently benefitted dancers' technique. As well as examining whether this effect was repeated in other dance cohorts and amenable to quantification within experimental outcomes, future investigations could also investigate DM-HIIT in other athlete populations, to assess whether responses to an acute training bout are different in acyclic athletes accustomed to multi-directional intermittent

activities, as observed in team sports, compared with athletes engaged in cyclic codes, such as running, where sagittal and continuous actions predominate.

In comparison with athlete populations, in which selective targeting of anaerobic metabolism may provide a functionally relevant and productive outcome, diverse exercise applied as HIIT may deliver no extra benefit in more general populations, where HIIT induced adaptations in aerobic metabolism have already been shown to provide significant improvement in health outcomes. For example, in patients with cardiovascular disease, HIIT exercise has been found to be superior to moderate-intensity continuous training, resulting in 9.1% higher increase in VO_2 peak for HIIT compared with MICT (Weston et al., 2014), and HIIT programmes targeting aerobic metabolism have resulted in measurable improvements in insulin sensitivity and high-density lipoprotein cholesterol (HDL-C) in > 12 w prescription (Kessler, Sisson, & Short, 2012). Although in its diverse format HIIT was well tolerated in sedentary females, both acutely and in longer-term application, and no adverse events were reported, there may be no additive effect of loading anaerobic metabolism by applying HIIT in the grid format, if improving cardiometabolic outcomes is the primary aim of exercise intervention in a non-athlete population. Nevertheless, the studies conducted do provide evidence that locomotor characteristics during exercise exhibited direction and threshold specific adaptations after longer term administration of brief, weekly bouts of diverse HIIT, which could be of potential osteogenic benefit in non-elite populations.

Whilst it is difficult to directly compare chronic effects of diverse exercise on locomotor profile across studies, as high threshold accelerations were not accessible during SSG, results for 12 weeks diverse HIIT in two distinct populations: pre-professional dance athletes and sedentary premenopausal females, followed the same trajectory of highly significant increases in vertical accelerations, with greatest increases above 3 g. The latter finding is critical in evaluating the extent to which studies reported here addressed the central question under examination: ‘is there an effect of brief, diverse exercise on bone?’. Preliminary evidence from Studies 3 and 4 suggests that after regular training dance athletes and sedentary females performed vertical accelerations more often, at higher thresholds and for longer during a matched bout of diverse exercise (Figure 42). What may be inferred from this, in answer to the principal

research question, is that locomotor profile exhibited direction and threshold specific adaptations towards an increase in accelerations demonstrated to elicit osteogenic effects (Ahola et al., 2010; Stiles et al., 2017), after chronic diverse HIIT in the novel format applied.

The evidence of bone anabolic effects for higher threshold accelerations is convincing and has been demonstrated both cross-sectionally, in diverse populations, and interventionally in different age groups. For example, exposure to daily accelerations above 3.6 g has been positively associated with regional increases in femoral BMD in females aged 35 - 40 (Jämsä et al., 2006), a finding corroborated for daily accelerations above 4 g in premenopausal females (Heikkinen et al., 2007), and in children and young adults, daily exposure to vigorous PA quantified by accelerometry was found to be an independent predictor of tibial bone strength (Kehrig, Björkman, Muhajarine, Johnston, & Kontulainen, 2019). Furthermore, assessment of vertical jumping has demonstrated strong, positive correlations between intensity of the accelerometer signal (according to thresholds and counts) and ground reaction force and peak loading rate (Rowlands & Stiles, 2012), and an osteogenic effect of jumping has been demonstrated longitudinally in young male athletes (Vlachopoulos, Barker, Ubago-Guisado, Williams, & Gracia-Marco, 2018). In Studies 3 and 4 no effect on calcaneal QUS was seen for chronic diverse HIIT, other than a tendency for left calcaneal stiffness index to be increased by 16% in male dancers, despite vertical accelerations increasing overall and at high threshold, whereas after 16 weeks SSG hip BMD significantly in sedentary females was increased whilst exercise intensity reduced.

These findings may be variously interpreted: given a timeline of ~4 – 6 months for bone adaptation (Clark, 2008), the relatively short duration of Studies 3 and 4 may explain lack of significant change in QUS for diverse HIIT, despite adaptation in locomotor profile showing an increase in higher-intensity accelerations, and therefore potential osteogenic effects, after chronic training. Whereas the increase observed in total hip BMD after 16 weeks SSG is at the lower limit of biological plausibility for quantifying an osteogenic effect of an exercise intervention, a limitation of the feasibility study (Chapter 5), and the training intervention in ballet dancers (Chapter 8), is low duration (12 weeks) in which to observe a bone anabolic effect. However, the two bout per week for 12

weeks training volume was dictated by access to dancers being limited to the duration of the ballet term (12 w), and there being only two free periods, within the dancers' weekly timetable, in which to schedule grid training. It was decided to match the overall (12 w) training volume in sedentary females (Chapter 7), to enable comparison between these interventions, and to match the duration of the initial investigation into feasibility and compliance with grid style HIIT (Chapter 5). Furthermore, overall volume of grid prescription at 3 (home exercise study, Chapter 7) or 2 (training intervention in dancers, Chapter 8) bouts per week may have been inadequate. For example, an osteogenic effect of exercise on hip and femoral BMD has been demonstrated in a weekly, dose-dependent manner for a jump exercise protocol (Bailey & Brooke-Wavell, 2010). Alternatively, no discernible effect on calcaneal bone for diverse HIIT may be attributable to limitations in resolution capacity for the method used to quantify bone adaptation, although QUS has been shown to exhibit fair to good agreement with DXA in evaluation of bone health in an adolescent population (Torres-Costoso et al., 2018).

A further aspect of the methodological approach to motion capture adopted in grid training studies, which could have affected interpretation of QUS in relation to locomotor profile, is the effect of MEMS sensor location. Evidence supports high functionality and wearability for placement of motion sensor units on the upper back (Gemperle, Kasabach, Stivoric, Bauer, & Martin, 1998), as undertaken in investigations described here, according to the rationale that most activities elicit trunk movement and the region represents the major part of body mass (Yang & Hsu, 2010). Whereas triaxial accelerometry data from trunk worn units have demonstrated high accuracy in estimating gait parameters (Moe-Nilssen & Helbostad, 2004), it cannot be discounted that positioning of the units on the lower limb, closer to the site measured directly by bone ultrasound, could have enhanced accuracy in representing accelerations directly associated with grid training and provided greater insight into the relationship between acceleration profile and bone outcomes. In future grid training studies, using ankle placement of sensors, as demonstrated to provide highly accurate representation of accelerations during gait (Yang & Hsu, 2010), could be explored to capture accelerations of distal limb segments directly measured by, for example, pQCT.

In relation to biomarkers of bone turnover, whilst background bone formation did not change after diverse exercise as either SSG or DM-HIIT, fasted CTX-1, a marker of bone resorption, reduced but only in female dancers after 12 weeks DM-HIIT, and increased steeply after three weeks detraining ($p < 0.001$). Although greater vertical accelerations were seen in all cohorts who trained for 12 weeks, the increase above 3 g in female dancers was large and whilst it may be speculated that diverse exercise as HIIT could have elicited an osteogenic effect by downregulating resorption in female dancers, further investigation is required to test this hypothesis. Furthermore, to assess whether diverse HIIT elicits acute elevation in markers of bone formation, as observed immediately after SSG in Study 1, sampling biomarkers post-exercise would enable upregulation of formation, if present after DM-HIIT, to be quantified.

9.3 Strengths and limitations of studies

The main strengths of studies undertaken were:

- development of a novel format of delivery for diverse exercise that replicated the demands of multi-directional team sports
- provision of evidence in two diverse populations – sedentary females and dancers- that the protocol achieved cardiometabolic thresholds described for HIIT, and selectively increased vertical high-threshold acceleration capacity during exercise
- demonstration in dancers and sedentary females of high acceptability and compliance with diverse HIIT in longer term training, providing evidence the protocol was accessible, acceptable and did not provoke adverse events
- flexible administration showed exercise could be undertaken both independently and under supervision
- provision of comprehensive and high quality data that delivered unique insights into bone metabolism, gonadal hormone and vitamin D status, both cross-sectionally and over a three-month period, in a hard to access dance population, in whom this has not previously been adequately described

Studies were limited by ecological factors, which constrained aspects of study design, and insufficient resources to gather comprehensive data sets

across all studies, which would have strengthened comparison of results and potential to generalise, with greater confidence, from conclusions that were drawn.

In Study 1 (RCT comparing brief bouts of small-sided football versus WBV), biomarkers of bone turnover were sampled both constitutively and acutely, after exercise exposure. Whilst in subsequent studies it would have been preferable to observe the same sampling schedule, to enable meaningful comparison and to characterise acute responses to diverse HIIT, we were either not resourced to do so financially (Grid Feasibility study, Chapter 5; Acute Physiological Effects of Grid Training, Chapter 6; Home Exercise Grid Training, Chapter 7; 12 Weeks Supplemental Grid Training in Dancers, Chapter 8), or this was not acceptable to stakeholders (Supplemental Grid Training in Dancers, Chapter 8). Furthermore, the Home Exercise Grid Training study described in Chapter 7 was not originally anticipated to form part of this thesis; the author contributed the grid protocol to enable comparison with a HIIT cycling regimen in this RCT, which investigated cardiometabolic adaptation to HIIT training. Its lead investigator provided CRP data, which was valuable in the context of inflammation and bone, however, sampling schedule and resources did not allow for acute post-exercise measurement of blood analytes, nor for background vitamin D to be assayed.

In the final study conducted in dancers, gonadal steroid hormones (GnH) and vitamin D were measured, alongside constitutive bone turnover markers, to provide comprehensive description of hormonal milieu and systemic context for bone turnover marker levels at baseline, and after 12 weeks of either grid supplemented or usual dance training. Moreover, whereas in a premenopausal female population, as investigated in small-sided football versus WBV and grid home exercise, cyclic fluctuations in eumenorrhoeic females limit the relevance of a single measurement to describe GnH status, evidence of hypothalamic-pituitary-gonadal dysregulation in ballet dancers indicated a requirement to assess GnHs, in order to pursue the central question of physiological adaptation and bone responses to exercise.

In summary, the most critical limitations were:

- low duration of interventions with diverse HIIT to assess bone change

- participant inaccessibility to post-exercise biomarker sampling (Studies 3 and 4) to quantify acute bone metabolic responses to exercise
- variation in methods used to measure bone for reasons of cost and location of research (Study 4)
- difference in accelerometer units and their position which was remote to the site of ground contact
- lack of quantification of forces during diverse HIIT impacts which would have enabled osteogenic loading index to be more accurately described

9.4 Application of findings and further research

The following recommendations and observations are made on the basis of evidence from Studies 3 and 4:

- diverse HIIT is an acceptable way to encourage sedentary females to exercise independently and provides a training stimulus, in brief format, that elicits a high anaerobic demand
- regular diverse HIIT may benefit athletes who participate in performance codes where multidirectional actions and intermittent demands on glycolytic pathways are high
- ballet dancers in pre-professional training, and stakeholders in dancers' health, should receive targeted advice about nutritional supplementation to address seasonal reduction in vitamin D
- altered levels of reproductive hormones observed in a sub-group of young female ballet dancers highlights a need for female dancers and those providing their healthcare to remain aware of the consequences on reproductive health and bone of menstrual dysregulation, possibly associated with an energy deficient state
- supplemental diverse HIIT may provide an osteogenic effect in young female dancers by reducing background bone resorption and inclining bone metabolism towards a net anabolic effect

Future controlled training studies with diverse HIIT should be conducted to examine the following questions:

- Is there an effect on femoral and tibial bone BMD after 6 months of training with diverse HIIT at a dose of 3 x 14 min per week in a sedentary population?

- Is there a quantifiable effect of 12 weeks diverse HIIT on technical ability in sports-specific task execution in athletes and does this vary between intermittent and continuous performance codes?
- Is there a role for DM-HIIT in bone injury prevention – such as reducing stress fracture incidence- in female dancers?
- Is diverse HIIT a feasible and acceptable approach to improve cardiometabolic fitness in older adults?

9.5 Conclusion

Diverse exercise, implemented in a HIIT format of constrained exercise area, was demonstrated to elicit high exercise heart rate and replicated anaerobic demands of intermittent team sports, in an athlete population and two cohorts of premenopausal sedentary females. However, further interventions are required to test the hypothesis that vertical accelerations, which predominantly characterise acceleration profile during diverse HIIT, provide an osteogenic stimulus in a longerterm (> 24 weeks) training scenario.

In future implementation, the effect of increasing exercise area on speed of movement during diverse HIIT could be examined, to assess whether this provides a stimulus to greater duration of accelerations at high threshold (> 3 g), and therefore enhances osteogenic potential for this approach to brief exercise.

Finally, restricting the prescription of exercise actions by featuring, for example, lateral jumping and progressions exclusively, and sampling bone biomarkers immediately after exposure, could enable responses to direction-dependent features of the movement signal to be characterised in a more quantal manner. This approach would enable greater resolution of the osteogenic signal than was provided here, during diverse HIIT, and increase specificity of prescription to include movement dose, in future exercise regimens aimed at improving bone health.

10 References

Abueidda, D. W., Sabet, F. A., & Jasiuk, I. M. (2017). Modeling of stiffness and strength of bone at nanoscale. *Journal of Biomechanical Engineering, 139*(5).

Ackerman, K. E., Nazem, T., Chapko, D., Russell, M., Mendes, N., Taylor, A. P., . . . Misra, M. (2011). Bone microarchitecture is impaired in adolescent amenorrheic athletes compared with eumenorrheic athletes and nonathletic controls. *The Journal of Clinical Endocrinology & Metabolism, 96*(10), 3123-3133.

Ackerman, K. E., Slusarz, K., Guereca, G., Pierce, L., Slattery, M., Mendes, N., . . . Misra, M. (2012). Higher ghrelin and lower leptin secretion are associated with lower LH secretion in young amenorrheic athletes compared with eumenorrheic athletes and controls. *American Journal of Physiology-Endocrinology and Metabolism, 302*(7), E800-E806.

Adam, C., Klissouras, V., & Ravasollo, M. (1988). Eurofit: Handbook for the Eurofit Tests of Physical Fitness: Rome: Council of Europe. *Committee for the Development of Sport*.

Adami, S., Giannini, S., Giorgino, R., Isaia, G. C., Maggi, S., Sinigaglia, L., . . . Crepaldi, G. (2004). Effect of Age, Weight and Lifestyle Factors on Calcaneal Quantitative Ultrasound in Premenopausal Women: The ESOPO Study. *Calcified tissue international, 74*(4), 317-321.

Adams, J. E. (2013). Advances in bone imaging for osteoporosis. *Nature Reviews Endocrinology, 9*(1), 28-42.

Aguiar, M., Botelho, G., Lago, C., Maças, V., & Sampaio, J. (2012). A review on the effects of soccer small-sided games. *Journal of human kinetics, 33*(2012), 103-113.

Ahola, R., Korpelainen, R., Vainionpää, A., & Jämsä, T. (2010). Daily impact score in long-term acceleration measurements of exercise. *Journal of biomechanics, 43*(10), 1960-1964.

Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., . . . Emplaincourt, P. O. (2000). Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and science in sports and exercise, 32*(9; SUPP/1), S498-S504.

Al-Hazzaa, H. M. (2018). Physical inactivity in Saudi Arabia revisited: a systematic review of inactivity prevalence and perceived barriers to active living. *International journal of health sciences, 12*(6), 50.

Ammann, P., & Rizzoli, R. (2003). Bone strength and its determinants. *Osteoporosis international, 14*(3), 13-18.

Amorim, T., Koutedakis, Y., Nevill, A., Wyon, M., Maia, J., Machado, J. C., . . . Dimitriou, L. (2017). Bone mineral density in vocational and professional ballet dancers. *Osteoporosis International, 28*(10), 2903-2912.

Amorim, T., Wyon, M., Maia, J., Machado, J. C., Marques, F., Metsios, G. S., . . . Koutedakis, Y. (2015). Prevalence of low bone mineral density in female dancers. *Sports Medicine, 45*(2), 257-268.

Angioi, M., Metsios, G., Koutedakis, Y., & Wyon, M. A. (2009). Fitness in contemporary dance: a systematic review. *International journal of sports medicine, 30*(07), 475-484.

Apazidis, A., Ricart, P. A., Diefenbach, C. M., & Spivak, J. M. (2011). The prevalence of transitional vertebrae in the lumbar spine. *The Spine Journal, 11*(9), 858-862.

Ashwell, M., Cole, T. J., & Dixon, A. K. (1996). Ratio of waist circumference to height is strong predictor of intra-abdominal fat. *Bmj, 313*(7056), 559-560.

Aughey, R. J. (2011). Applications of GPS technologies to field sports. *International journal of sports physiology and performance, 6*(3), 295-310.

Babatunde, O. O., & Forsyth, J. J. (2013). Quantitative Ultrasound and bone's response to exercise: A meta analysis. *Bone, 53*(1), 311-318.

Bacchi, E., Spiazzi, G., Zendrini, G., Bonin, C., & Moghetti, P. (2013). Low body weight and menstrual dysfunction are common findings in both elite and amateur ballet dancers. *Journal of Endocrinological Investigation, 36*(5), 343-346.

Bailey, C. A., & Brooke-Wavell, K. (2010). Optimum frequency of exercise for bone health: randomised controlled trial of a high-impact unilateral intervention. *Bone, 46*(4), 1043-1049.

Baim, S., Wilson, C. R., Lewiecki, E. M., Luckey, M. M., Downs Jr, R. W., & Lentle, B. C. (2005). Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry. *Journal of Clinical Densitometry, 8*(4), 371-378.

Baker, J. F., Davis, M., Alexander, R., Zemel, B. S., Mostoufi-Moab, S., Shults, J., . . . Leonard, M. B. (2013). Associations between body composition and bone density and structure in men and women across the adult age spectrum. *Bone, 53*(1), 34-41.

Ball, K., Salmon, J., Giles-corti, B., & Crawford, D. (2006). How Can Socio-Economic Differences in Physical Activity Among Women Be Explained? A Qualitative Study. *Women & Health, 43*(1), 93-113.

Bangsbo, J. (2015). Performance in sports--With specific emphasis on the effect of intensified training. *Scand J Med Sci Sports, 25 Suppl 4*, 88-99.

Bangsbo, J., Junge, A., Dvorak, J., & Krustrup, P. (2014). Executive summary: Football for health – prevention and treatment of non-communicable diseases across the lifespan through football. *Scandinavian Journal of Medicine & Science in Sports, 24*(S1), 147-150.

Bangsbo, J., Mohr, M., & Krustrup, P. (2006). Physical and metabolic demands of training and match-play in the elite football player. *Journal of sports sciences, 24*(07), 665-674.

Bangsbo, J., Nielsen, J. J., Mohr, M., Randers, M. B., Krustrup, B. R., Brito, J., . . . Krustrup, P. (2010). Performance enhancements and muscular adaptations of a 16-week recreational football intervention for untrained women. *Scandinavian journal of medicine & science in sports, 20*, 24-30.

Barene, S., Krustrup, P., Jackman, S., Brekke, O., & Holtermann, A. (2014). Do soccer and Z umba exercise improve fitness and indicators of health among female hospital employees? A 12-week RCT. *Scandinavian journal of medicine & science in sports, 24*(6), 990-999.

Barrett, S., Midgley, A., & Lovell, R. (2014). PlayerLoad™: reliability, convergent validity, and influence of unit position during treadmill running. *International journal of sports physiology and performance, 9*(6), 945-952.

Barrett, S., Midgley, A. W., Towlson, C., Garrett, A., Portas, M., & Lovell, R. (2016). Within-match PlayerLoad™ patterns during a simulated soccer match: potential implications for unit positioning and fatigue management. *International journal of sports physiology and performance, 11*(1), 135-140.

Bartl, R., & Bartl, C. (2019). Definition of Osteoporosis. In R. Bartl & C. Bartl (Eds.), *The Osteoporosis Manual: Prevention, Diagnosis and Management* (pp. 93-95). Cham: Springer International Publishing.

Bastard, J.-P., Maachi, M., Lagathu, C., Kim, M. J., Caron, M., Vidal, H., . . . Feve, B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network, 17*(1), 4-12.

Bautista, L., Reininger, B., Gay, J. L., Barroso, C. S., & McCormick, J. B. (2011). Perceived barriers to exercise in Hispanic adults by level of activity. *Journal of physical activity & health, 8*(7), 916-925.

Beck, S., Redding, E., & Wyon, M. A. (2015). Methodological considerations for documenting the energy demand of dance activity: a review. *Frontiers in psychology, 6*, 568.

Bellver, M., Del Rio, L., Jovell, E., Drobnic, F., & Trilla, A. (2019). Bone mineral density and bone mineral content among female elite athletes. *Bone, 127*, 393-400.

Biddle, S. J. H., & Batterham, A. M. (2015). High-intensity interval exercise training for public health: a big HIT or shall we HIT it on the head?

International Journal of Behavioral Nutrition and Physical Activity, 12(1), 95.

Bidwell, J. P., Alvarez, M. B., Hood, M., & Childress, P. (2013). Functional impairment of bone formation in the pathogenesis of osteoporosis: the bone marrow regenerative competence. *Current osteoporosis reports, 11*(2), 117-125.

Bischoff-Ferrari, H. (2009). Vitamin D: what is an adequate vitamin D level and how much supplementation is necessary? *Best Practice & Research Clinical Rheumatology, 23*(6), 789-795.

Bischoff-Ferrari, H. A., Willett, W. C., Wong, J. B., Giovannucci, E., Dietrich, T., & Dawson-Hughes, B. (2005). Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *Jama, 293*(18), 2257-2264.

Blake, G. M., & Fogelman, I. (2007). The role of DXA bone density scans in the diagnosis and treatment of osteoporosis. *Postgraduate medical journal, 83*(982), 509-517.

Bonaventura, J. M., Sharpe, K., Knight, E., Fuller, K. L., Tanner, R. K., & Gore, C. J. (2015). Reliability and accuracy of six hand-held blood lactate analysers. *Journal of sports science & medicine, 14*(1), 203-214.

Bonewald, L. (2019). Use it or lose it to age: A review of bone and muscle communication. *Bone, 120*, 212-218.

Bonewald, L. F., & Johnson, M. L. (2008). Osteocytes, mechanosensing and Wnt signaling. *Bone, 42*(4), 606-615.

Boonen, S., Pye, S. R., O'Neill, T. W., Szulc, P., Gielen, E., Borghs, H., . . . Ward, K. A. (2011). Influence of bone remodelling rate on quantitative ultrasound parameters at the calcaneus and DXA BMDa of the hip and spine in middle-aged and elderly European men: the European Male Ageing Study (EMAS). *European journal of endocrinology, 165*(6), 977.

Booth, S. L., Centi, A., Smith, S. R., & Gundberg, C. (2013). The role of osteocalcin in human glucose metabolism: marker or mediator? *Nature Reviews Endocrinology, 9*(1), 43-55.

Borg, E., & Kaijser, L. (2006a). A comparison between three rating scales for perceived exertion and two different work tests. *Scandinavian journal of medicine & science in sports, 16*(1), 57-69.

Borg, E., & Kaijser, L. (2006b). A comparison between three rating scales for perceived exertion and two different work tests. *Scand J Med Sci Sports, 16*(1), 57-69.

Bourdon, P. C., Cardinale, M., Murray, A., Gastin, P., Kellmann, M., Varley, M. C., . . . Gregson, W. (2017). Monitoring athlete training loads: consensus statement. *International journal of sports physiology and performance, 12*(s2), S2-161-S162-170.

Bowtell, J. L., Jackman, S. R., Scott, S., Connolly, L. J., Mohr, M., Ermidis, G., . . . Jørgensen, N. R. (2016). Short duration small sided football and to a lesser extent whole body vibration exercise induce acute changes in markers of bone turnover. *BioMed Research International, 2016*.

Boyce, B. F., & Xing, L. (2008). Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys, 473*(2), 139-146.

Boyd, L. J., Ball, K., & Aughey, R. J. (2011). The reliability of MinimaxX accelerometers for measuring physical activity in Australian football. *International journal of sports physiology and performance, 6*(3), 311-321.

Bradley, P. S., Mohr, M., Bendiksen, M., Randers, M., Flindt, M., Barnes, C., . . . Di Mascio, M. (2011). Sub-maximal and maximal Yo–Yo intermittent endurance test level 2: heart rate response, reproducibility and application to elite soccer. *European journal of applied physiology, 111*(6), 969-978.

Bradley, P. S., Sheldon, W., Wooster, B., Olsen, P., Boanas, P., & Krustrup, P. (2009). High-intensity running in English FA Premier League soccer matches. *Journal of sports sciences, 27*(2), 159-168.

Bravo-Sánchez, A., Abián-Vicén, J., Jiménez, F., & Abián, P. (2020). Influence of badminton practice on calcaneal bone stiffness and plantar pressure. *The Physician and Sportsmedicine, 48*(1), 98-104.

Bria, S., Bianco, M., Galvani, C., Palmieri, V., Zeppilli, P., & Faina, M. (2011). Physiological characteristics of elite sport-dancers. *The Journal of sports medicine and physical fitness, 51*(2), 194-203.

Brito, J., Krustrup, P., & Rebelo, A. (2012). The influence of the playing surface on the exercise intensity of small-sided recreational soccer games. *Human Movement Science, 31*(4), 946-956.

Brown, M. A., Howatson, G., Quin, E., Redding, E., & Stevenson, E. J. (2017). Energy intake and energy expenditure of pre-professional female contemporary dancers. *PloS one, 12*(2), e0171998.

Buchheit, M., Haydar, B., Hader, K., Ufland, P., & Ahmaidi, S. (2011). Assessing running economy during field running with changes of direction: application to 20 m shuttle runs. *International journal of sports physiology and performance, 6*(3), 380-395.

Buchheit, M., & Laursen, P. B. (2013). High-intensity interval training, solutions to the programming puzzle. *Sports medicine, 43*(10), 927-954.

Buchheit, M., Mendez-Villanueva, A., Simpson, B., & Bourdon, P. (2010). Match running performance and fitness in youth soccer. *International journal of sports medicine, 31*(11), 818-825.

Bujalance-Moreno, P., Latorre-Román, P. Á., & García-Pinillos, F. (2019). A systematic review on small-sided games in football players: Acute and chronic adaptations. *Journal of sports sciences, 37*(8), 921-949.

Burr, D. (2003). Microdamage and bone strength. *Osteoporosis International, 14*(5), 67-72.

Burr, D. B. (1997). Muscle strength, bone mass, and age-related bone loss. *Journal of Bone and Mineral Research, 12*(10), 1547-1551.

Burr, D. B., Milgrom, C., Fyhrie, D., Forwood, M., Nyska, M., Finestone, A., . . . Simkin, A. (1996). In vivo measurement of human tibial strains during vigorous activity. *Bone, 18*(5), 405-410.

Burr, D. B., Ruff, C. B., & Johnson, C. (1989). Structural adaptations of the femur and humerus to arboreal and terrestrial environments in three species of macaque. *American Journal of Physical Anthropology, 79*(3), 357-367.

Byrne, H., Caulfield, B., & De Vito, G. (2018). Self-directed exercise programmes in sedentary middle-aged individuals in good overall health; a systematic review. *Prev Med, 114*, 156-163.

Cabell, L., Pienkowski, D., Shapiro, R., & Janura, M. (2013). Effect of age and activity level on lower extremity gait dynamics: an introductory study. *J Strength Cond Res, 27*(6), 1503-1510.

Callegari, E. T., Gorelik, A., Garland, S. M., Chiang, C. Y., & Wark, J. D. (2017). Bone turnover marker reference intervals in young females. *Annals of clinical biochemistry, 54*(4), 438-447.

Camomilla, V., Bergamini, E., Fantozzi, S., & Vannozzi, G. (2018). Trends supporting the in-field use of wearable inertial sensors for sport performance evaluation: A systematic review. *Sensors, 18*(3), 873.

Campbell, W. W., Kraus, W. E., Powell, K. E., Haskell, W. L., Janz, K. F., Jakicic, J. M., . . . Piercy, K. L. (2019). High-intensity interval training for cardiometabolic disease prevention. *Medicine & Science in Sports & Exercise, 51*(6), 1220-1226.

Cao, J. J. (2011). Effects of obesity on bone metabolism. *Journal of orthopaedic surgery and research, 6*(1), 30.

Capodaglio, E. (2001). Comparison between the CR10 Borg's scale and the VAS (visual analogue scale) during an arm-cranking exercise. *Journal of occupational rehabilitation, 11*(2), 69-74.

Carbuhn, A. F., Fernandez, T. E., Bragg, A. F., Green, J. S., & Crouse, S. F. (2010). Sport and training influence bone and body composition in women collegiate athletes. *The Journal of Strength & Conditioning Research, 24*(7), 1710-1717.

Casamichana, D., Castellano, J., & Castagna, C. (2012). Comparing the Physical Demands of Friendly Matches and Small-Sided Games in Semiprofessional Soccer Players. *The Journal of Strength & Conditioning Research, 26*(3).

Castagna, C., D'Ottavio, S., & Abt, G. (2003). Activity profile of young soccer players during actual match play. *The Journal of Strength & Conditioning Research, 17*(4), 775-780.

Castagna, C., Impellizzeri, F. M., Belardinelli, R., Abt, G., COUTTS, A., CHAMARI, K., & D'OTTAVIO, S. (2006). Cardiorespiratory responses to Yo-yo Intermittent Endurance Test in nonelite youth soccer players. *The Journal of Strength & Conditioning Research, 20*(2), 326-330.

Cavani, F., Giavaresi, G., Fini, M., Bertoni, L., De Terlizzi, F., Barkmann, R., & Can, V. (2008). Influence of density, elasticity, and structure on ultrasound transmission through trabecular bone cylinders. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control, 55*(7), 1465-1472.

Chastin, S. F., Mandrichenko, O., Helbostadt, J., & Skelton, D. A. (2014). Associations between objectively-measured sedentary behaviour and physical activity with bone mineral density in adults and older adults, the NHANES study. *Bone, 64*, 254-262.

Chen, M. J., Fan, X., & Moe, S. T. (2002). Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. *Journal of sports sciences, 20*(11), 873-899.

Chen, M. M., Lear, S. A., Gao, M., Frohlich, J. J., & Birmingham, C. L. (2001). Intraobserver and interobserver reliability of waist circumference and the waist-to-hip ratio. *Obesity, 9*(10), 651.

Cheng, S., Sipilä, S., Taaffe, D., Puolakka, J., & Suominen, H. (2002). Change in bone mass distribution induced by hormone replacement therapy and high-impact physical exercise in post-menopausal women. *Bone, 31*(1), 126-135.

Chin, K. Y., & Ima-Nirwana, S. (2013). Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect? *Int J Med Sci, 10*(12), 1778-1783.

Christensen, B., Dandanell, S., Kjaer, M., & Langberg, H. (2011). Effect of anti-inflammatory medication on the running-induced rise in patella tendon collagen synthesis in humans. *Journal of applied physiology, 110*(1), 137-141.

Church, T. S., Thomas, D. M., Tudor-Locke, C., Katzmarzyk, P. T., Earnest, C. P., Rodarte, R. Q., . . . Bouchard, C. (2011). Trends over 5 decades in US occupation-related physical activity and their associations with obesity. *PloS one, 6*(5), e19657.

Cianferotti, L., & Brandi, M. L. (2014). Muscle–bone interactions: basic and clinical aspects. *Endocrine, 45*(2), 165-177.

Civil, R., Lamb, A., Loosmore, D., Ross, L., Livingstone, K., Strachan, F., . . . Witard, O. C. (2019). Assessment of dietary intake, energy status, and factors associated with RED-S in vocational female ballet students. *Frontiers in Nutrition, 5*, 136.

Claessens, A. L., Beunen, G. P., Nuyts, M. M., Lefevre, J., & Wellens, R. (1987). Body structure, somatotype, maturation and motor performance of girls in ballet schooling. *J Sports Med, 27*, 310-317.

Clark, B. (2008). Normal bone anatomy and physiology. *Clin J Am Soc Nephrol, 3*(Supl 3), S131-S139.

Clissold, T. L., Cronin, J. B., De Souza, M. J., Wilson, D., & Winwood, P. W. (2019). Bilateral multidirectional jumps with reactive jump-landings achieve osteogenic thresholds with and without instruction in premenopausal women. *Clin Biomech (Bristol, Avon), 73*, 1-8.

Cohen, J. L., Segal, K. R., & McArdle, W. D. (1982). Heart rate response to ballet stage performance. *The Physician and Sportsmedicine, 10*(11), 120-133.

Cohen, J. L., Segal, K. R., Witriol, I., & McArdle, W. D. (1982). Cardiorespiratory responses to ballet exercise and the VO2max of elite ballet dancers. *Medicine and science in sports and exercise, 14*(3), 212-217.

Colaianni, G., Cuscito, C., Mongelli, T., Pignataro, P., Buccoliero, C., Liu, P., . . . Mori, G. (2015). The myokine irisin increases cortical bone mass. *Proceedings of the National Academy of Sciences, 112*(39), 12157-12162.

Colaianni, G., Mongelli, T., Colucci, S., Cinti, S., & Grano, M. (2016). Crosstalk Between Muscle and Bone Via the Muscle-Myokine Irisin. *Current Osteoporosis Reports, 14*(4), 132-137.

Colley, R. C., Hills, A. P., O'Moore-Sullivan, T. M., Hickman, I. J., Prins, J. B., & Byrne, N. M. (2008). Variability in adherence to an unsupervised exercise prescription in obese women. *International journal of obesity, 32*(5), 837-844.

Confavreux, C. B., Levine, R. L., & Karsenty, G. (2009). A paradigm of integrative physiology, the crosstalk between bone and energy metabolisms. *Molecular and cellular endocrinology, 310*(1-2), 21-29.

Connolly, L. J., Scott, S., Mohr, M., Ermidis, G., Julian, R., Bangsbo, J., . . . Hopkins, S. J. (2014). Effects of small-volume soccer and vibration training on body composition, aerobic fitness, and muscular PCr kinetics for inactive women aged 20–45. *Journal of Sport and Health Science, 3*(4), 284-292.

Connolly, L. J., Scott, S., Morencos, C. M., Fulford, J., Jones, A. M., Knapp, K., . . . Bowtell, J. L. (2020). Impact of a novel home-based exercise intervention on health indicators in inactive premenopausal women: a 12-week randomised controlled trial. *European journal of applied physiology, 120*(4), 771-782.

Consultation, W. (2008). Waist circumference and waist-hip ratio. *Report of a WHO Expert Consultation. Geneva: World Health Organization, 2008*, 8-11.

Cornier, M.-A., Despres, J.-P., Davis, N., Grossniklaus, D. A., Klein, S., Lamarche, B., . . . Towfighi, A. (2011). Assessing adiposity: a scientific statement from the American Heart Association. *Circulation, 124*(18), 1996-2019.

Crameri, R. M., Langberg, H., Teisner, B., Magnusson, P., Schrøder, H. D., Olesen, J. L., . . . Kjaer, M. (2004). Enhanced procollagen processing in skeletal muscle after a single bout of eccentric loading in humans. *Matrix biology, 23*(4), 259-264.

Cummins, C., Orr, R., O'Connor, H., & West, C. (2013). Global positioning systems (GPS) and microtechnology sensors in team sports: a systematic review. *Sports Med, 43*(10), 1025-1042.

Currey, J. D. (2003). The many adaptations of bone. *Journal of Biomechanics, 36*(10), 1487-1495.

Da Shao, H., Li, G. W., Liu, Y., Qiu, Y. Y., Yao, J. H., & Tang, G. Y. (2015). Contributions of fat mass and fat distribution to hip bone strength in healthy postmenopausal Chinese women. *Journal of bone and mineral metabolism, 33*(5), 507-515.

Dalen, T., Jørgen, I., Gertjan, E., Geir Havard, H., & Ulrik, W. (2016). Player Load, Acceleration, and Deceleration During Forty-Five Competitive Matches of Elite Soccer. *The Journal of Strength & Conditioning Research, 30*(2).

Damilakis, J., Adams, J. E., Guglielmi, G., & Link, T. M. (2010). Radiation exposure in X-ray-based imaging techniques used in osteoporosis. *European radiology, 20*(11), 2707-2714.

Dandona, P., Aljada, A., & Bandyopadhyay, A. (2004). Inflammation: the link between insulin resistance, obesity and diabetes. *Trends in Immunology, 25*(1), 4-7.

De Lorenzo, A., Gratteri, S., Gualtieri, P., Cammarano, A., Bertucci, P., & Di Renzo, L. (2019). Why primary obesity is a disease? *Journal of translational medicine, 17*(1), 1-13.

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

De Souza, M. J., Lee, D. K., VanHeest, J. L., Scheid, J. L., West, S. L., & Williams, N. I. (2007). Severity of energy-related menstrual disturbances increases in proportion to indices of energy conservation in exercising women. *Fertility and sterility, 88*(4), 971-975.

De Souza, M. J., West, S. L., Jamal, S. A., Hawker, G. A., Gundberg, C. M., & Williams, N. I. (2008). The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone, 43*(1), 140-148.

De Souza, M. J., & Williams, N. I. (2004). Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women. *Human reproduction update, 10*(5), 433-448.

Deere, K., Sayers, A., Rittweger, J., & Tobias, J. H. (2012). A cross-sectional study of the relationship between cortical bone and high-impact activity in young adult males and females. *J Clin Endocrinol Metab, 97*(10), 3734-3743.

Dekker, J., Nelson, K., Kurgan, N., Falk, B., Josse, A., & Klentrou, P. (2017). Wnt signaling–related osteokines and transforming growth factors before and after a single bout of plyometric exercise in child and adolescent females. *Pediatric exercise science, 29*(4), 504-512.

Dellal, A., Hill-Haas, S., Lago-Penas, C., & Chamari, K. (2011). Small-sided games in soccer: amateur vs. professional players' physiological responses, physical, and technical activities. *The Journal of Strength & Conditioning Research, 25*(9), 2371-2381.

Dellal, A., Keller, D., Carling, C., Chaouachi, A., Wong, d. P., & Chamari, K. (2010). Physiologic effects of directional changes in intermittent exercise in soccer players. *The Journal of Strength & Conditioning Research, 24*(12), 3219-3226.

DeLuccia, R., Cheung, M., Ramadoss, R., Aljahdali, A., & Sukumar, D. (2019). The Endocrine Role of Bone in Cardiometabolic Health. *Current Nutrition Reports, 8*(3), 281-294.

Demerath, E. W., Shen, W., Lee, M., Choh, A. C., Czerwinski, S. A., Siervogel, R. M., & Towne, B. (2007). Approximation of total visceral adipose tissue with a single magnetic resonance image. *The American journal of clinical nutrition, 85*(2), 362-368.

Dolan, E., Dumas, A., Keane, K. M., Bestetti, G., Freitas, L. H. M., Gualano, B., . . . Swinton, P. (2020). The influence of acute exercise on bone biomarkers: protocol for a systematic review with meta-analysis. *Systematic Reviews, 9*(1), 291.

Dolan, E., McGoldrick, A., Davenport, C., Kelleher, G., Byrne, B., Tormey, W., . . . Warrington, G. D. (2012). An altered hormonal profile and elevated rate of bone loss are associated with low bone mass in professional horse-racing jockeys. *Journal of bone and mineral metabolism, 30*(5), 534-542.

Dolny, D. G., & Reyes, G. F. C. (2008). Whole body vibration exercise: training and benefits. *Current sports medicine reports, 7*(3), 152-157.

Dowd, K. P., Szeklicki, R., Minetto, M. A., Murphy, M. H., Polito, A., Ghigo, E., . . . Donnelly, A. E. (2018). A systematic literature review of reviews on techniques for physical activity measurement in adults: a DEDIPAC study. *International Journal of Behavioral Nutrition and Physical Activity, 15*(1), 15.

Dowthwaite, J. N., Rosenbaum, P. F., & Scerpella, T. A. (2011). Mechanical loading during growth is associated with plane-specific differences in vertebral geometry: a cross-sectional analysis comparing artistic gymnasts vs. non-gymnasts. *Bone, 49*(5), 1046-1054.

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

Eckel, R. H., Grundy, S. M., & Zimmet, P. Z. (2005). The metabolic syndrome. *The Lancet, 365*(9468), 1415-1428.

Ehrampoush, E., Arasteh, P., Homayounfar, R., Cheraghpour, M., Alipour, M., Naghizadeh, M. M., . . . Razaz, J. M. (2017). New anthropometric indices or old ones: Which is the better predictor of body fat? *Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 11*(4), 257-263.

Elliott-Sale, K. J., Tenforde, A. S., Parziale, A. L., Holtzman, B., & Ackerman, K. E. (2018). Endocrine Effects of Relative Energy Deficiency in Sport. *Int J Sport Nutr Exerc Metab, 28*(4), 335-349.

Ellis, K. J. (2000). Human Body Composition: In Vivo Methods. *Physiological Reviews, 80*(2), 649-680.

Elmlinger, M. W., Kühnel, W., & Ranke, M. B. (2002). Reference ranges for serum concentrations of lutropin (LH), follitropin (FSH), estradiol (E2), prolactin, progesterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and ferritin in neonates, children and young adults. *Clinical Chemistry and Laboratory Medicine, 40*(11), 1151-1160.

Engel, F. A., Ackermann, A., Chtourou, H., & Sperlich, B. (2018). High-Intensity Interval Training Performed by Young Athletes: A Systematic Review and Meta-Analysis. *Frontiers in Physiology, 9*(1012).

Ericksen, H. M., Gribble, P. A., Pfile, K. R., & Pietrosimone, B. G. (2013). Different modes of feedback and peak vertical ground reaction force during jump landing: a systematic review. *J Athl Train, 48*(5), 685-695.

Faienza, M. F., D'Amato, G., Chiarito, M., Colaianni, G., Colucci, S., Grano, M., . . . Brunetti, G. (2019). Mechanisms involved in childhood obesity-related bone fragility. *Frontiers in endocrinology, 10*, 269.

Fernandes, T. A. P., Gonçalves, L. M. L., & Brito, J. A. A. (2017). Relationships between Bone Turnover and Energy Metabolism. *Journal of Diabetes Research, 2017*, 9021314.

Figueroa, A., Going, S. B., Milliken, L. A., Blew, R. M., Sharp, S., Teixeira, P. J., & Lohman, T. G. (2003). Effects of Exercise Training and Hormone Replacement Therapy on Lean and Fat Mass in Postmenopausal Women. *The Journals of Gerontology: Series A, 58*(3), M266-M270.

Fleet, J. C. (2017). The role of vitamin D in the endocrinology controlling calcium homeostasis. *Molecular and cellular endocrinology, 453*, 36-45.

Fonseca, H., Moreira-Gonçalves, D., Coriolano, H.-J. A., & Duarte, J. A. (2014). Bone quality: the determinants of bone strength and fragility. *Sports medicine, 44*(1), 37-53.

Foxdal, P., Bergqvist, Y., Eckerbom, S., & Sandhagen, B. (1992). Improving lactate analysis with the YSI 2300 GL: hemolyzing blood samples makes results comparable with those for deproteinized whole blood. *Clinical Chemistry, 38*(10), 2110-2114.

Fredericson, M., Bergman, A. G., Hoffman, K. L., & Dillingham, M. S. (1995). Tibial stress reaction in runners: correlation of clinical symptoms and scintigraphy with a new magnetic resonance imaging grading system. *The American journal of sports medicine, 23*(4), 472-481.

Fredericson, M., Chew, K., Ngo, J., Cleek, T., Kiratli, J., & Cobb, K. (2007). Regional bone mineral density in male athletes: a comparison of soccer players, runners and controls. *British journal of sports medicine, 41*(10), 664-668.

Freitas, L., Amorim, T., Humbert, L., Fonollá, R., Flouris, A. D., Metsios, G. S., . . . Koutedakis, Y. (2019). Cortical and trabecular bone analysis of professional dancers using 3D-DXA: a case–control study. *Journal of sports sciences, 37*(1), 82-89.

Frost, H. M. (1987). Bone “mass” and the “mechanostat”: a proposal. *The anatomical record, 219*(1), 1-9.

Frost, H. M. (2004). A 2003 update of bone physiology and Wolff's Law for clinicians. *The Angle Orthodontist, 74*(1), 3-15.

Fu, X., Ma, X., Lu, H., He, W., Wang, Z., & Zhu, S. (2011). Associations of fat mass and fat distribution with bone mineral density in pre-and postmenopausal Chinese women. *Osteoporosis international, 22*(1), 113-119.

Fukuda, T. Y., Melo, W. P., Zaffalon, B. M., Rossetto, F. M., Magalhaes, E., Bryk, F. F., & Martin, R. L. (2012). Hip posterolateral musculature strengthening in sedentary women with patellofemoral pain syndrome: a randomized controlled clinical trial with 1-year follow-up. *J Orthop Sports Phys Ther, 42*(10), 823-830.

Gabbett, T. J., & Mulvey, M. J. (2008). Time-motion analysis of small-sided training games and competition in elite women soccer players. *The Journal of Strength & Conditioning Research, 22*(2), 543-552.

Gallagher, D., Visser, M., Sepulveda, D., Pierson, R. N., Harris, T., & Heymsfield, S. B. (1996). How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *American journal of epidemiology, 143*(3), 228-239.

Gardinier, J. D., Mohamed, F., & Kohn, D. H. (2015). PTH signaling during exercise contributes to bone adaptation. *Journal of Bone and Mineral Research, 30*(6), 1053-1063.

Garnero, P. (2017). The utility of biomarkers in osteoporosis management. *Molecular diagnosis & therapy, 21*(4), 401-418.

Gemperle, F., Kasabach, C., Stivoric, J., Bauer, M., & Martin, R. (1998). *Design for wearability.* Paper presented at the digest of papers. Second international symposium on wearable computers (cat. No. 98EX215).

Gibala, M. J. (2018). Interval Training for Cardiometabolic Health: Why Such A HIIT? *Current Sports Medicine Reports, 17*(5).

Gibala, M. J., Gillen, J. B., & Percival, M. E. (2014). Physiological and Health-Related Adaptations to Low-Volume Interval Training: Influences of Nutrition and Sex. *Sports Medicine, 44*(2), 127-137.

Gibala, M. J., Little, J. P., MacDonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *The Journal of physiology, 590*(5), 1077-1084.

Gibala, M. J., & McGee, S. L. (2008). Metabolic adaptations to short-term high-intensity interval training: a little pain for a lot of gain? *Exercise and sport sciences reviews, 36*(2), 58-63.

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., & Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nature reviews immunology, 11*(9), 607-615.

Gliemann, L., Gunnarsson, T. P., Hellsten, Y., & Bangsbo, J. (2015). 10-20-30 training increases performance and lowers blood pressure and VEGF in runners. *Scand J Med Sci Sports, 25*(5), e479-489.

Gluer, C., Wu, C., Jergas, M., Goldstein, S., & Genant, H. (1994). Three quantitative ultrasound parameters reflect bone structure. *Calcified tissue international, 55*(1), 46-52.

Going, S., Lohman, T., Houtkooper, L., Metcalfe, L., Flint-Wagner, H., Blew, R., . . . Weber, J. (2003). Effects of exercise on bone mineral density in calcium-replete postmenopausal women with and without hormone replacement therapy. *Osteoporosis International, 14*(8), 637-643.

Grace, F., Herbert, P., Elliott, A. D., Richards, J., Beaumont, A., & Sculthorpe, N. F. (2018). High intensity interval training (HIIT) improves resting blood pressure, metabolic (MET) capacity and heart rate reserve without compromising cardiac function in sedentary aging men. *Experimental Gerontology, 109*, 75-81.

Grisendi, V., Spada, E., Argento, C., Plebani, M., Milani, S., Seracchioli, R., . . . La Marca, A. (2014). Age-specific reference values for serum FSH and estradiol levels throughout the reproductive period. *Gynecol Endocrinol, 30*(6), 451-455.

Gross, T., & Nelson, R. C. (1988). The shock attenuation role of the ankle during landing from a vertical jump. *Medicine and science in sports and exercise, 20*(5), 506-514.

Gross, T. S., Edwards, J. L., Mcleod, K. J., & Rubin, C. T. (1997). Strain gradients correlate with sites of periosteal bone formation. *Journal of Bone and Mineral Research, 12*(6), 982-988.

Guadalupe-Grau, A., Fuentes, T., Guerra, B., & Calbet, J. A. L. (2009). Exercise and Bone Mass in Adults. *Sports Medicine, 39*(6), 439-468.

Guglielmi, V., & Sbraccia, P. (2018). Obesity phenotypes: depot-differences in adipose tissue and their clinical implications. *Eating and weight Disorders-Studies on anorexia, bulimia and obesity, 23*(1), 3-14.

Guidetti, L., Gallotta, M., Emerenziani, G. P., & Baldari, C. (2007). Exercise intensities during a ballet lesson in female adolescents with different technical ability. *International journal of sports medicine, 28*(09), 736-742.

Gunnarsson, T. P., & Bangsbo, J. (2012). The 10-20-30 training concept improves performance and health profile in moderately trained runners. *Journal of Applied Physiology, 113*(1), 16-24.

Guri, A. J., & Bassaganya-Riera, J. (2011). Systemic Effects of White Adipose Tissue Dysregulation and Obesity-Related Inflammation. *Obesity, 19*(4), 689-700.

Guy-Cherry, D., Alanazi, A., Miller, L., Staloch, D., & Ortiz-Rodriguez, A. (2018). Landing Styles Influences Reactive Strength Index without Increasing Risk for Injury. *Sports Med Int Open, 2*(2), E35-e40.

Hadji, P., Hars, O., Wüster, C., Bock, K., Alberts, U. S., Bohnet, H. G., . . . Schulz, K. D. (1999). Stiffness index identifies patients with osteoporotic fractures better than ultrasound velocity or attenuation alone. *Maturitas, 31*(3), 221-226.

Hadjidakis, D. J., & Androulakis, I. I. (2006). Bone remodeling. *Annals of the New York Academy of Sciences, 1092*(1), 385-396.

Hagman, M., Helge, E. W., Hornstrup, T., Fristrup, B., Nielsen, J. J., Jørgensen, N. R., . . . Krustrup, P. (2018). Bone mineral density in lifelong trained male football players compared with young and elderly untrained men. *Journal of sport and health science, 7*(2), 159-168.

Haight, H. J. (1998). Morphologic, physiologic, and functional interactions in elite female ballet dancers. *Medical Problems of Performing Artists, 13*, 4-13.

Hallal, P. C., Andersen, L. B., Bull, F. C., Guthold, R., Haskell, W., & Ekelund, U. (2012). Global physical activity levels: surveillance progress, pitfalls, and prospects. *The Lancet, 380*(9838), 247-257.

Halouani, J., Chtourou, H., Gabbett, T., Chaouachi, A., & Chamari, K. (2014). Small-sided games in team sports training: a brief review. *The journal of strength & conditioning research, 28*(12), 3594-3618.

Hamilton, B. (2010). Vitamin D and human skeletal muscle. *Scandinavian journal of medicine & science in sports, 20*(2), 182-190.

Hangartner, T. N., Warner, S., Braillon, P., Jankowski, L., & Shepherd, J. (2013). The Official Positions of the International Society for Clinical Densitometry: acquisition of dual-energy X-ray absorptiometry body composition and considerations regarding analysis and repeatability of measures. *Journal of Clinical Densitometry, 16*(4), 520-536.

Hannam, K., Deere, K., Hartley, A., Clark, E., Coulson, J., Ireland, A., . . . Gaysin, T. (2017). A novel accelerometer-based method to describe day-to-day exposure to potentially osteogenic vertical impacts in older adults: findings from a multi-cohort study. *Osteoporosis International, 28*(3), 1001-1011.

Hans, D., & Baim, S. (2017). Quantitative Ultrasound (QUS) in the Management of Osteoporosis and Assessment of Fracture Risk. *J Clin Densitom, 20*(3), 322-333.

Harding, A. T., & Beck, B. R. (2017). Exercise, osteoporosis, and bone geometry. *Sports, 5*(2), 29.

Hardouin, P., Pansini, V., & Cortet, B. (2014). Bone marrow fat. *Joint Bone Spine, 81*(4), 313-319.

Harper, D. J., Carling, C., & Kiely, J. (2019). High-intensity acceleration and deceleration demands in elite team sports competitive match play: A systematic review and meta-analysis of observational studies. *Sports Medicine*, 1-25.

Hart, N. H., Nimphius, S., Rantalainen, T., Ireland, A., Siafarikas, A., & Newton, R. (2017). Mechanical basis of bone strength: influence of bone material, bone structure and muscle action. *Journal of musculoskeletal & neuronal interactions, 17*(3), 114.

Hatamoto, Y., Yamada, Y., Sagayama, H., Higaki, Y., Kiyonaga, A., & Tanaka, H. (2014). The relationship between running velocity and the energy cost of turning during running. *PloS one, 9*(1), e81850.

Heikkinen, R., Vihriala, E., Vainionpaa, A., Korpelainen, R., & Jamsa, T. (2007). Acceleration slope of exercise-induced impacts is a determinant of changes in bone density. *J Biomech, 40*(13), 2967-2974.

Heinonen, A., Mäntynen, J., Kannus, P., Uusi-Rasi, K., Nikander, R., Kontulainen, S., & Sievänen, H. (2012). Effects of high-impact training and detraining on femoral neck structure in premenopausal women: a hip structural analysis of an 18-month randomized controlled exercise intervention with 3.5-year follow-up. *Physiotherapy Canada. Physiotherapie Canada, 64*(1), 98-105.

Helge, E. W., Aagaard, P., Jakobsen, M. D., Sundstrup, E., Randers, M. B., Karlsson, M. K., & Krustrup, P. (2010). Recreational football training decreases risk factors for bone fractures in untrained premenopausal women. *Scandinavian journal of medicine & science in sports, 20*, 31-39.

Helge, E. W., Andersen, T. R., Schmidt, J. F., Jørgensen, N. R., Hornstrup, T., Krustrup, P., & Bangsbo, J. (2014). Recreational football improves bone mineral density and bone turnover marker profile in elderly men. *Scandinavian journal of medicine & science in sports, 24*, 98-104.

Helge, E. W., & Kanstrup, I.-L. (2002). Bone density in female elite gymnasts: impact of muscle strength and sex hormones. *Medicine and science in sports and exercise, 34*(1), 174-180.

Hernandez, C. J., & van der Meulen, M. C. (2017). Understanding bone strength is not enough. *Journal of Bone and Mineral Research, 32*(6), 1157-1162.

Heron, L., Neill, C., McAneney, H., Kee, F., & Tully, M. A. (2019). Direct healthcare costs of sedentary behaviour in the UK. *Journal of Epidemiology and Community Health, 73*(7), 625.

Heymsfield, S. B., Peterson, C. M., Thomas, D. M., Heo, M., & Schuna Jr, J. (2016). Why are there race/ethnic differences in adult body mass index–adiposity relationships? A quantitative critical review. *Obesity reviews, 17*(3), 262-275.

Hill-Haas, S. V., Dawson, B., Impellizzeri, F. M., & Coutts, A. J. (2011). Physiology of small-sided games training in football. *Sports medicine, 41*(3), 199-220.

Hincapié, C. A., & Cassidy, J. D. (2010). Disordered eating, menstrual disturbances, and low bone mineral density in dancers: a systematic review. *Archives of Physical Medicine and Rehabilitation, 91*(11), 1777-1789. e1771.

Hinrichs, T., Chae, E.-H., Lehmann, R., Allolio, B., & Platen, P. (2010). Bone mineral density in athletes of different disciplines: a cross-sectional study. *The Open Sports Sciences Journal, 3*(1).

Hinton, P. V., Rackard, S. M., & Kennedy, O. D. (2018). In Vivo Osteocyte Mechanotransduction: Recent Developments and Future Directions. *Current Osteoporosis Reports, 16*(6), 746-753.

Hoch, A. Z., Papanek, P., Szabo, A., Widlansky, M. E., Schimke, J. E., & Gutterman, D. D. (2011). Association between the female athlete triad and endothelial dysfunction in dancers. *Clinical journal of sport medicine: official journal of the Canadian Academy of Sport Medicine, 21*(2), 119.

Hocking, S., Samocha-Bonet, D., Milner, K.-L., Greenfield, J. R., & Chisholm, D. J. (2013). Adiposity and insulin resistance in humans: the role of the different tissue and cellular lipid depots. *Endocrine reviews, 34*(4), 463-500.

Hojman, P., Gehl, J., Christensen, J. F., & Pedersen, B. K. (2018). Molecular mechanisms linking exercise to cancer prevention and treatment. *Cell metabolism, 27*(1), 10-21.

Hood, K., Ashcraft, J., Watts, K., Hong, S., Choi, W., Heymsfield, S. B., . . . Thomas, D. (2019). Allometric scaling of weight to height and resulting body mass index thresholds in two Asian populations. *Nutrition & Diabetes, 9*(1), 2.

Hornstrup, T., Wikman, J. M., Fristrup, B., Póvoas, S., Helge, E. W., Nielsen, S. H., . . . Krustrup, P. (2018). Fitness and health benefits of team handball training for young untrained women—A cross-disciplinary RCT on physiological adaptations and motivational aspects. *Journal of Sport and Health Science, 7*(2), 139-148.

Hu, H. H., Chen, J., & Shen, W. (2016). Segmentation and quantification of adipose tissue by magnetic resonance imaging. *Magnetic Resonance Materials in Physics, Biology and Medicine, 29*(2), 259-276.

Hummel, T., Delwiche, J. F., Schmidt, C., & Hüttenbrink, K.-B. (2003). Effects of the form of glasses on the perception of wine flavors: a study in untrained subjects. *Appetite, 41*(2), 197-202.

Idolazzi, L., El Ghoch, M., Dalle Grave, R., Bazzani, P., Calugi, S., Fassio, S., . . . Braga, V. (2018). Bone metabolism in patients with anorexia nervosa and amenorrhoea. *Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity, 23*(2), 255-261.

Iida, T., Chikamura, C., Aoi, S., Ikeda, H., Matsuda, Y., Oguri, Y., . . . Ishizaki, F. (2010). A study on the validity of quantitative ultrasonic measurement used the bone mineral density values on dual-energy X-ray absorptiometry in young and in middle-aged or older women. *Radiological physics and technology, 3*(2), 113-119.

Imboden, M. T., Welch, W. A., Swartz, A. M., Montoye, A. H. K., Finch, H. W., Harber, M. P., & Kaminsky, L. A. (2017). Reference standards for body fat measures using GE dual energy x-ray absorptiometry in Caucasian adults. *PloS one, 12*(4), e0175110-e0175110.

Ireland, A., Sayers, A., Deere, K. C., Emond, A., & Tobias, J. H. (2016). Motor Competence in Early Childhood Is Positively Associated With Bone Strength in Late Adolescence. *J Bone Miner Res, 31*(5), 1089-1098.

Irlbeck, T., Massaro, J. M., Bamberg, F., O'Donnell, C. J., Hoffmann, U., & Fox, C. S. (2010). Association between single-slice measurements of visceral and abdominal subcutaneous adipose tissue with volumetric measurements: the Framingham Heart Study. *International journal of obesity (2005), 34*(4), 781-787.

Ito, S. (2019). High-intensity interval training for health benefits and care of cardiac diseases - The key to an efficient exercise protocol. *World J Cardiol, 11*(7), 171-188.

Jackman, S. R., Scott, S., Randers, M. B., Ørntoft, C., Blackwell, J., Zar, A., . . . Krustrup, P. (2013). Musculoskeletal health profile for elite female footballers versus untrained young women before and after 16 weeks of football training. *Journal of Sports Sciences, 31*(13), 1468-1474.

Jackson, A. S., Stanforth, P. R., Gagnon, J., Rankinen, T., Leon, A. S., Rao, D. C., . . . Wilmore, J. H. (2002). The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. *International Journal of Obesity, 26*(6), 789-796.

Jain, S., & Camacho, P. (2018). Use of bone turnover markers in the management of osteoporosis. *Current Opinion in Endocrinology, Diabetes and Obesity, 25*(6), 366-372.

Jämsä, T., Ahola, R., & Korpelainen, R. (2011). Measurement of osteogenic exercise–how to interpret accelerometric data? *Frontiers in physiology, 2*, 73.

Jämsä, T., Vainionpää, A., Korpelainen, R., Vihriälä, E., & Leppäluoto, J. (2006). Effect of daily physical activity on proximal femur. *Clinical Biomechanics, 21*(1), 1-7.

Jelleyman, C., Yates, T., O'Donovan, G., Gray, L. J., King, J. A., Khunti, K., & Davies, M. J. (2015). The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. *Obesity reviews, 16*(11), 942-961.

Jennings, D., Cormack, S., Coutts, A. J., Boyd, L., & Aughey, R. J. (2010a). The validity and reliability of GPS units for measuring distance in team sport specific running patterns. *International journal of sports physiology and performance, 5*(3), 328-341.

Jennings, D., Cormack, S., Coutts, A. J., Boyd, L. J., & Aughey, R. J. (2010b). Variability of GPS units for measuring distance in team sport movements. *International journal of sports physiology and performance, 5*(4), 565-569.

Judex, S., Gross, T. S., & Zernicke, R. F. (1997). Strain gradients correlate with sites of exercise-induced bone-forming surfaces in the adult skeleton. *Journal of Bone and Mineral Research, 12*(10), 1737-1745.

Judex, S., & Zernicke, R. F. (2000). High-impact exercise and growing bone: relation between high strain rates and enhanced bone formation. *Journal of applied physiology, 88*(6), 2183-2191.

Jürimäe, J. (2010). Interpretation and application of bone turnover markers in children and adolescents. *Current opinion in pediatrics, 22*(4), 494-500.

Kambas, A., Leontsini, D., Avloniti, A., Chatzinikolaou, A., Stampoulis, T., Makris, K., . . . Fatouros, I. G. (2017). Physical activity may be a potent regulator of bone turnover biomarkers in healthy girls during preadolescence. *Journal of Bone and Mineral Metabolism, 35*(6), 598-607.

Kanazawa, I. (2015). Osteocalcin as a hormone regulating glucose metabolism. *World journal of diabetes, 6*(18), 1345.

Karantonis, D. M., Narayanan, M. R., Mathie, M., Lovell, N. H., & Celler, B. G. (2006). Implementation of a real-time human movement classifier using a triaxial accelerometer for ambulatory monitoring. *IEEE transactions on information technology in biomedicine, 10*(1), 156-167.

Karcher, C., & Buchheit, M. (2014). On-Court Demands of Elite Handball, with Special Reference to Playing Positions. *Sports Medicine, 44*(6), 797-814.

Kato, T., Terashima, T., Yamashita, T., Hatanaka, Y., Honda, A., & Umemura, Y. (2006). Effect of low-repetition jump training on bone mineral density in young women. *J Appl Physiol (1985), 100*(3), 839-843.

Kaul, S., Rothney, M. P., Peters, D. M., Wacker, W. K., Davis, C. E., Shapiro, M. D., & Ergun, D. L. (2012). Dual-energy X-ray absorptiometry for quantification of visceral fat. *Obesity, 20*(6), 1313-1318.

Keating, S., Johnson, N., Mielke, G., & Coombes, J. (2017). A systematic review and meta-analysis of interval training versus moderate-intensity continuous training on body adiposity. *Obesity reviews, 18*(8), 943-964.

Kehrig, A. M., Björkman, K. M., Muhajarine, N., Johnston, J. D., & Kontulainen, S. A. (2019). Moderate to vigorous physical activity and impact loading independently predict variance in bone strength at the tibia but not at the radius in children. *Applied Physiology, Nutrition, and Metabolism, 44*(3), 326-331.

Kendler, D. L., Borges, J. L., Fielding, R. A., Itabashi, A., Krueger, D., Mulligan, K., . . . Elaine, W. Y. (2013). The official positions of the International Society for Clinical Densitometry: indications of use and reporting of DXA for body composition. *Journal of Clinical Densitometry, 16*(4), 496-507.

Kenkre, J., & Bassett, J. (2018). The bone remodelling cycle. *Annals of clinical biochemistry, 55*(3), 308-327.

Kessler, H. S., Sisson, S. B., & Short, K. R. (2012). The potential for high-intensity interval training to reduce cardiometabolic disease risk. *Sports medicine, 42*(6), 489-509.

Kim, H.-J., Koo, H. S., Kim, Y.-S., Kim, M. J., Kim, K.-M., Joo, N.-S., & Haam, J.-h. (2017). The association of testosterone, sex hormone-binding globulin, and insulin-like growth factor-1 with bone parameters in Korean men aged 50 years or older. *Journal of bone and mineral metabolism, 35*(6), 659-665.

Kim, H., Lee, S., Kim, B., & Koh, J. (2017). Association between obesity and femoral neck strength according to age, sex, and fat distribution. *Osteoporosis International, 28*(7), 2137-2146.

Kim, J.-E., Hsieh, M.-H., Soni, B. K., Zayzafoon, M., & Allison, D. B. (2013). Childhood obesity as a risk factor for bone fracture: A mechanistic study. *Obesity, 21*(7), 1459-1466.

Kindblom, J. M., Ohlsson, C., Ljunggren, Ö., Karlsson, M. K., Tivesten, Å., Smith, U., & Mellström, D. (2009). Plasma Osteocalcin Is Inversely Related to Fat Mass and Plasma Glucose in Elderly Swedish Men. *Journal of Bone and Mineral Research, 24*(5), 785-791.

Kish, K., Mezil, Y., Ward, W. E., Klentrou, P., & Falk, B. (2015). Effects of plyometric exercise session on markers of bone turnover in boys and young men. *European journal of applied physiology, 115*(10), 2115-2124.

Koehler, K., Hoerner, N. R., Gibbs, J. C., Zinner, C., Braun, H., De Souza, M. J., & Schaenzer, W. (2016). Low energy availability in exercising men is associated with reduced leptin and insulin but not with changes in other metabolic hormones. *Journal of Sports Sciences, 34*(20), 1921-1929.

Kohn, D. H., Sahar, N. D., Wallace, J. M., Golcuk, K., & Morris, M. D. (2009). Exercise alters mineral and matrix composition in the absence of adding new bone. *Cells Tissues Organs, 189*(1-4), 33-37.

Kohrt, W. M., Ehsani, A. A., & Birge JR, S. J. (1997). Effects of exercise involving predominantly either joint-reaction or ground-reaction forces on bone mineral density in older women. *Journal of Bone and Mineral Research, 12*(8), 1253-1261.

Kohrt, W. M., Wherry, S. J., Wolfe, P., Sherk, V. D., Wellington, T., Swanson, C. M., . . . Boxer, R. S. (2018). Maintenance of serum ionized calcium during exercise attenuates parathyroid hormone and bone resorption responses. *Journal of Bone and Mineral Research, 33*(7), 1326-1334.

Koltun, K. J., De Souza, M. J., Scheid, J. L., & Williams, N. I. (2020). Energy availability is associated with luteinizing hormone pulse frequency and induction of luteal phase defects. *The Journal of Clinical Endocrinology & Metabolism, 105*(1), 185-193.

Komori, T. (2006). Regulation of osteoblast differentiation by transcription factors. *Journal of cellular biochemistry, 99*(5), 1233-1239.

Kong, Z., Fan, X., Sun, S., Song, L., Shi, Q., & Nie, J. (2016). Comparison of high-intensity interval training and moderate-to-vigorous continuous training for cardiometabolic health and exercise enjoyment in obese young women: a randomized controlled trial. *PloS one, 11*(7), e0158589.

Koutedakis, Y., & Jamurtas, A. (2004). The dancer as a performing athlete. *Sports medicine, 34*(10), 651-661.

Kouvelioti, R., Kurgan, N., Falk, B., Ward, W. E., Josse, A. R., & Klentrou, P. (2018). Response of Sclerostin and Bone Turnover Markers to High Intensity Interval Exercise in Young Women: Does Impact Matter? *Biomed Res Int, 2018*, 4864952.

Kranti Panta, B. (2015). A study to associate the Flamingo Test and the Stork Test in measuring static balance on healthy adults.

Krieg, M.-A., Barkmann, R., Gonnelli, S., Stewart, A., Bauer, D. C., Barquero, L. D. R., . . . Olszynski, W. P. (2008). Quantitative ultrasound in the

management of osteoporosis: the 2007 ISCD Official Positions. *Journal of Clinical Densitometry, 11*(1), 163-187.

Krustrup, P., Aagaard, P., Nybo, L., Petersen, J., Mohr, M., & Bangsbo, J. (2010). Recreational football as a health promoting activity: a topical review. *Scandinavian Journal of Medicine & Science in Sports, 20*(s1), 1-13.

Krustrup, P., Christensen, J. F., Randers, M. B., Pedersen, H., Sundstrup, E., Jakobsen, M. D., . . . Nybo, L. (2010). Muscle adaptations and performance enhancements of soccer training for untrained men. *European journal of applied physiology, 108*(6), 1247-1258.

Krustrup, P., Hansen, P. R., Andersen, L. J., Jakobsen, M. D., Sundstrup, E., Randers, M. B., . . . Søgaard, P. (2010). Long-term musculoskeletal and cardiac health effects of recreational football and running for premenopausal women. *Scandinavian journal of medicine & science in sports, 20*, 58-71.

Krustrup, P., Helge, E. W., Hansen, P. R., Aagaard, P., Hagman, M., Randers, M. B., . . . Mohr, M. (2018). Effects of recreational football on women's fitness and health: adaptations and mechanisms. *European journal of applied physiology, 118*(1), 11-32.

Krustrup, P., Williams, C., Mohr, M., Hansen, P., Helge, E., Elbe, A. M., . . . Hammami, A. (2018). The "Football is Medicine" platform—scientific evidence, large-scale implementation of evidence-based concepts and future perspectives. *Scandinavian Journal of Medicine & Science in Sports, 28*, 3-7.

Książek, A., Zagrodna, A., & Słowińska-Lisowska, M. (2019). Vitamin D, skeletal muscle function and athletic performance in athletes—A narrative review. *Nutrients, 11*(8), 1800.

Kujala, U. M. (2009). Evidence on the effects of exercise therapy in the treatment of chronic disease. *British Journal of Sports Medicine, 43*(8), 550-555.

Lamberts, R. P., Swart, J., Noakes, T. D., & Lambert, M. I. (2011). A novel submaximal cycle test to monitor fatigue and predict cycling performance. *British journal of sports medicine, 45*(10), 797-804.

Lanyon, L. E., & Rubin, C. (1984). Static vs dynamic loads as an influence on bone remodelling. *Journal of biomechanics, 17*(12), 897-905.

Larsson, P. (2003). Global positioning system and sport-specific testing. *Sports Medicine, 33*(15), 1093-1101.

Lee, I. M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N., Katzmarzyk, P. T., & Lancet Physical Activity Series Working, G. (2012). Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet (London, England), 380*(9838), 219-229.

Lee, K., Jessop, H., Suswillo, R., Zaman, G., & Lanyon, L. (2003). Bone adaptation requires oestrogen receptor-α. *Nature, 424*(6947), 389-389.

Lee, S., & Lee, D. K. (2018). What is the proper way to apply the multiple comparison test? *Korean journal of anesthesiology, 71*(5), 353.

Lee, S. W., Jo, H. H., Kim, M. R., Kim, J. H., & You, Y. O. (2015). Association between osteocalcin and metabolic syndrome in postmenopausal women. *Archives of Gynecology and Obstetrics, 292*(3), 673-681.

Lester, M. E., Urso, M. L., Evans, R. K., Pierce, J. R., Spiering, B. A., Maresh, C. M., . . . Nindl, B. C. (2009). Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. *Bone, 45*(4), 768-776.

Lewitt, M. S. (2017). The role of the growth hormone/insulin-like growth factor system in visceral adiposity. *Biochemistry insights, 10*, 1178626417703995.

Lienhard, K., Vienneau, J., Nigg, S., Friesenbichler, B., & Nigg, B. M. (2017). Older adults show higher increases in lower-limb muscle activity during whole-body vibration exercise. *Journal of biomechanics, 52*, 55-60.

Liiv, H., Wyon, M. A., Jürimäe, T., Saar, M., Mäestu, J., & Jürimäe, J. (2013). Anthropometry, somatotypes, and aerobic power in ballet, contemporary dance, and dancesport. *Medical problems of performing artists, 28*(4), 207-211.

Liu, J. (2015). Irisin as an exercise-stimulated hormone binding crosstalk between organs. *Eur Rev Med Pharmacol Sci, 19*(2), 316-321.

Lombardi, G. (2019). Exercise-Dependent Modulation of Bone Metabolism and Bone Endocrine Function: New Findings and Therapeutic Perspectives. *Journal of Science in Sport and Exercise, 1*(1), 20-28.

Lombardi, G., Perego, S., Luzi, L., & Banfi, G. (2015). A four-season molecule: osteocalcin. Updates in its physiological roles. *Endocrine, 48*(2), 394-404.

Loucks, A. B., & Thuma, J. R. (2003). Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *The Journal of Clinical Endocrinology & Metabolism, 88*(1), 297-311.

Lozano-Berges, G., Matute-Llorente, Á., Gómez-Bruton, A., González-Agüero, A., Vicente-Rodriguez, G., & Casajús, J. A. (2018). Bone geometry in young male and female football players: a peripheral quantitative computed tomography (pQCT) study. *Archives of osteoporosis, 13*(1), 57.

Lu, T.-W., Taylor, S. J. G., O'Connor, J. J., & Walker, P. S. (1997). Influence of muscle activity on the forces in the femur: An in vivo study. *Journal of Biomechanics, 30*(11), 1101-1106.

Lucey, A. J., Paschos, G. K., Thorsdottir, I., Martínéz, J. A., Cashman, K. D., & Kiely, M. (2013). Young overweight and obese women with lower circulating osteocalcin concentrations exhibit higher insulin resistance and concentrations of C-reactive protein. *Nutrition Research, 33*(1), 67-75.

Luo, H., Newton, R. U., Ma'ayah, F., Galvão, D. A., & Taaffe, D. R. (2018). Recreational soccer as sport medicine for middle-aged and older adults: a systematic review. *BMJ Open Sport Exerc Med, 4*(1), e000336.

Ma, C., Tonks, K., Center, J., Samocha-Bonet, D., & Greenfield, J. (2018). Complex interplay among adiposity, insulin resistance and bone health. *Clinical obesity, 8*(2), 131-139.

Madou, K. H., & Cronin, J. B. (2008). The effects of whole body vibration on physical and physiological capability in special populations. *Hong Kong Physiotherapy Journal, 26*(1), 24-38.

Maïmoun, L., Garnero, P., Mura, T., Nocca, D., Lefebvre, P., Philibert, P., . . . Courtet, P. (2020). Specific effects of anorexia nervosa and obesity on bone mineral density and bone turnover in young women. *The Journal of Clinical Endocrinology & Metabolism, 105*(4), e1536-e1548.

Maimoun, L., Manetta, J., Couret, I., Dupuy, A., Mariano-Goulart, D., Micallef, J., . . . Rossi, M. (2006). The intensity level of physical exercise and the bone metabolism response. *International journal of sports medicine, 27*(02), 105-111.

Maldonado, G., Soueres, P., & Watier, B. (2018). Strategies of Parkour practitioners for executing soft precision landings. *J Sports Sci, 36*(22), 2551-2557.

Malina, R. M., Claessens, A. L., Van Aken, K., Thomis, M., Lefevre, J., Philippaerts, R., & Beunen, G. P. (2006). Maturity offset in gymnasts: application of a prediction equation. *Medicine and science in sports and exercise, 38*(7), 1342-1347.

Manhard, M. K., Nyman, J. S., & Does, M. D. (2017). Advances in imaging approaches to fracture risk evaluation. *Translational research : the journal of laboratory and clinical medicine, 181*, 1-14.

Marędziak, M., Śmieszek, A., Chrząstek, K., Basinska, K., & Marycz, K. (2015). Physical activity increases the total number of bone-marrow-derived mesenchymal stem cells, enhances their osteogenic potential, and inhibits their adipogenic properties. *Stem Cells International, 2015*.

Martyn-St James, M., & Carroll, S. (2009). A meta-analysis of impact exercise on postmenopausal bone loss: the case for mixed loading exercise programmes. *British journal of sports medicine, 43*(12), 898.

Martyn-St James, M., & Carroll, S. (2010). Effects of different impact exercise modalities on bone mineral density in premenopausal women: a meta-analysis. *Journal of bone and mineral metabolism, 28*(3), 251-267.

Maruyama-Nagao, A., Sakuraba, K., & Suzuki, Y. (2016). Seasonal variations in vitamin D status in indoor and outdoor female athletes. *Biomedical reports, 5*(1), 113-117.

Marwaha, R. K., Tandon, N., Reddy, D. R. H., Aggarwal, R., Singh, R., Sawhney, R. C., . . . Singh, S. (2005). Vitamin D and bone mineral density status of healthy schoolchildren in northern India–. *The American journal of clinical nutrition, 82*(2), 477-482.

Matsukura, T., Kagamimori, S., Nishino, H., Yamagami, T., Iki, M., Kajita, E., . . . Marumo, F. (2003). The characteristics of bone turnover in the second decade in relation to age and puberty development in healthy Japanese male and female subjects-Japanese Population-based Osteoporosis Study. *Annals of human biology, 30*(1), 13-25.

Matthew, D., & Delextrat, A. (2009). Heart rate, blood lactate concentration, and time–motion analysis of female basketball players during competition. *Journal of sports sciences, 27*(8), 813-821.

McCormack, M. C., Bird, H., de Medici, A., Haddad, F., & Simmonds, J. (2019). The Physical Attributes Most Required in Professional Ballet: A Delphi Study. *Sports Med Int Open, 3*(1), E1-e5.

McGee, S. L., & Hargreaves, M. (2020). Exercise adaptations: molecular mechanisms and potential targets for therapeutic benefit. *Nature Reviews Endocrinology*, 1-11.

McIver, V., Greig, M., & Marrin, K. (2019). The physiological and mechanical demands of aerobics, indoor cycling, and netball in recreationally active females. *Sport Sciences for Health, 15*(3), 535-541.

Meakin, L. B., Price, J. S., & Lanyon, L. E. (2014). The contribution of experimental in vivo models to understanding the mechanisms of adaptation to mechanical loading in bone. *Frontiers in endocrinology, 5*, 154.

Meeuwsen, S., Horgan, G., & Elia, M. (2010). The relationship between BMI and percent body fat, measured by bioelectrical impedance, in a large adult sample is curvilinear and influenced by age and sex. *Clinical nutrition, 29*(5), 560-566.

Menz, V., Marterer, N., Amin, S. B., Faulhaber, M., Hansen, A. B., & Lawley, J. S. (2019). Functional Vs. Running Low-Volume High-Intensity Interval Training: Effects on VO(2)max and Muscular Endurance. *Journal of sports science & medicine, 18*(3), 497-504.

Meredith-Jones, K., Haszard, J., Stanger, N., & Taylor, R. (2018). Precision of DXA-derived visceral fat measurements in a large sample of adults of varying body size. *Obesity, 26*(3), 505-512.

Milanović, Z., Pantelić, S., Čović, N., Sporiš, G., Mohr, M., & Krustrup, P. (2019). Broad-spectrum physical fitness benefits of recreational football: a systematic review and meta-analysis. *British journal of sports medicine, 53*(15), 926-939.

Miller, W. G. (2017). *Glucose Analytical Comparability Evaluation of the YSI 2300 STAT PlusTM and YSI 2900D Biochemistry Analyzers*.

Mitsiopoulos, N., Baumgartner, R. N., Heymsfield, S. B., Lyons, W., Gallagher, D., & Ross, R. (1998). Cadaver validation of skeletal muscle measurement by magnetic resonance imaging and computerized tomography. *Journal of Applied Physiology, 85*(1), 115-122.

Moe-Nilssen, R., & Helbostad, J. L. (2004). Estimation of gait cycle characteristics by trunk accelerometry. *Journal of biomechanics, 37*(1), 121-126.

Mohr, M., Helge, E. W., Petersen, L. F., Lindenskov, A., Weihe, P., Mortensen, J., . . . Krustrup, P. (2015). Effects of soccer vs swim training on bone formation in sedentary middle-aged women. *European journal of applied physiology, 115*(12), 2671-2679.

Monteiro, R., & Azevedo, I. (2010). Chronic Inflammation in Obesity and the Metabolic Syndrome. *Mediators of Inflammation, 2010*, 289645.

Moore, S. A., McKay, H. A., Macdonald, H., Nettlefold, L., Baxter-Jones, A. D., Cameron, N., & Brasher, P. M. (2015). Enhancing a somatic maturity prediction model. *Medicine & Science in Sports & Exercise, 47*(8), 1755-1764.

Morgan, W. P. (1994). Psychological components of effort sense. *Medicine & Science in Sports & Exercise*.

Mountjoy, M., Sundgot-Borgen, J., Burke, L., Carter, S., Constantini, N., Lebrun, C., . . . Budgett, R. (2014). The IOC consensus statement: beyond the female athlete triad—relative energy deficiency in sport (RED-S). *British journal of sports medicine, 48*(7), 491-497.

Mountjoy, M., Sundgot-Borgen, J. K., Burke, L. M., Ackerman, K. E., Blauwet, C., Constantini, N., . . . Meyer, N. L. (2018). IOC consensus statement on relative energy deficiency in sport (RED-S): 2018 update. *British journal of sports medicine*.

Movahed, A., Larijani, B., Nabipour, I., Kalantarhormozi, M., Asadipooya, K., Vahdat, K., . . . Amirinejad, R. (2012). Reduced serum osteocalcin concentrations are associated with type 2 diabetes mellitus and the metabolic syndrome components in postmenopausal women: the crosstalk between bone and energy metabolism. *Journal of bone and mineral metabolism, 30*(6), 683-691.

Mudd, L. M., Fornetti, W., & Pivarnik, J. M. (2007). Bone mineral density in collegiate female athletes: comparisons among sports. *Journal of athletic training, 42*(3), 403.

Myers, A., Gibbons, C., Finlayson, G., & Blundell, J. (2017). Associations among sedentary and active behaviours, body fat and appetite dysregulation: investigating the myth of physical inactivity and obesity. *British journal of sports medicine, 51*(21), 1540.

Myers, C. A., Torry, M. R., Peterson, D. S., Shelburne, K. B., Giphart, J. E., Krong, J. P., . . . Steadman, J. R. (2011). Measurements of tibiofemoral kinematics during soft and stiff drop landings using biplane fluoroscopy. *Am J Sports Med, 39*(8), 1714-1722.

Nana, A., Slater, G. J., Hopkins, W. G., & Burke, L. M. (2013). Effects of exercise sessions on DXA measurements of body composition in active people. *Medicine & Science in Sports & Exercise, 45*(1), 178-185.

Narra, N., Nikander, R., Viik, J., Hyttinen, J., & Sievänen, H. (2013). Femoral neck cross-sectional geometry and exercise loading. *Clinical physiology and functional imaging, 33*(4), 258-266.

National Institute for Health and Care Excellence. (2020). Vitamin D deficiency in adults-treatment and prevention.

Nedergaard, N. J., Robinson, M. A., Eusterwiemann, E., Drust, B., Lisboa, P. J., & Vanrenterghem, J. (2017). The relationship between whole-body external loading and body-worn accelerometry during team-sport movements. *International journal of sports physiology and performance, 12*(1), 18-26.

Neufer, P. D., Bamman, Marcas M., Muoio, Deborah M., Bouchard, C., Cooper, Dan M., Goodpaster, Bret H., . . . Laughlin, Maren R. (2015). Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits. *Cell Metabolism, 22*(1), 4-11.

Nigg, B. M., & Yeadon, M. R. (1987). Biomechanical aspects of playing surfaces. *Journal of Sports Sciences, 5*(2), 117-145.

Nikander, R., Sievänen, H., Heinonen, A., Daly, R. M., Uusi-Rasi, K., & Kannus, P. (2010). Targeted exercise against osteoporosis: a systematic review and meta-analysis for optimising bone strength throughout life. *BMC medicine, 8*(1), 47.

Nikander, R., Sievänen, H., Heinonen, A., & Kannus, P. (2005). Femoral neck structure in adult female athletes subjected to different loading modalities. *Journal of Bone and Mineral Research, 20*(3), 520-528.

Niu, K., Ahola, R., Guo, H., Korpelainen, R., Uchimaru, J., Vainionpää, A., . . . Kishimoto, K. (2010). Effect of office-based brief high-impact exercise on bone mineral density in healthy premenopausal women: the Sendai Bone Health Concept Study. *Journal of bone and mineral metabolism, 28*(5), 568-577.

Niu, W., Feng, T., Jiang, C., & Zhang, M. (2014). Peak vertical ground reaction force during two-leg landing: a systematic review and mathematical modeling. *Biomed Res Int, 2014*, 126860.

Njeh, C., Hans, D., Li, J., Fan, B., Fuerst, T., He, Y., . . . Genant, H. (2000). Comparison of six calcaneal quantitative ultrasound devices: precision and hip fracture discrimination. *Osteoporosis International, 11*(12), 1051-1062.

Noonan, V., & Dean, E. (2000). Submaximal exercise testing: clinical application and interpretation. *Physical therapy, 80*(8), 782-807.

Nordhamn, K., Södergren, E., Olsson, E., Karlström, B., Vessby, B., & Berglund, L. (2000). Reliability of anthropometric measurements in overweight and lean subjects: consequences for correlations between anthropometric and other variables. *International Journal of Obesity, 24*(5), 652-657.

Norton, K., Norton, L., & Sadgrove, D. (2010). Position statement on physical activity and exercise intensity terminology. *Journal of Science and Medicine in Sport, 13*(5), 496-502.

Oftadeh, R., Perez-Viloria, M., Villa-Camacho, J. C., Vaziri, A., & Nazarian, A. (2015). Biomechanics and mechanobiology of trabecular bone: a review. *J Biomech Eng, 137*(1), 0108021-01080215.

Oja, P., Titze, S., Kokko, S., Kujala, U. M., Heinonen, A., Kelly, P., . . . Foster, C. (2015). Health benefits of different sport disciplines for adults: systematic review of observational and intervention studies with meta-analysis. *British journal of sports medicine, 49*(7), 434-440.

Okazaki, R., Inoue, D., Shibata, M., Saika, M., Kido, S., Ooka, H., . . . Matsumoto, T. (2002). Estrogen promotes early osteoblast differentiation and inhibits adipocyte differentiation in mouse bone marrow stromal cell lines that express estrogen receptor (ER) α or β. *Endocrinology, 143*(6), 2349-2356.

Olney, N., Wertz, T., LaPorta, Z., Mora, A., Serbas, J., & Astorino, T. A. (2018). Comparison of acute physiological and psychological responses between moderate-intensity continuous exercise and three regimes of high-intensity interval training. *The Journal of Strength & Conditioning Research, 32*(8), 2130-2138.

Ormsbee, M. J., Prado, C. M., Ilich, J. Z., Purcell, S., Siervo, M., Folsom, A., & Panton, L. (2014). Osteosarcopenic obesity: the role of bone, muscle, and fat on health. *Journal of cachexia, sarcopenia and muscle, 5*(3), 183-192.

Ott, S. M. (2018). Cortical or trabecular bone: what's the difference? *American journal of nephrology, 47*(6), 373-376.

Owen, N., Healy, G. N., Matthews, C. E., & Dunstan, D. W. (2010). Too Much Sitting: The Population Health Science of Sedentary Behavior. *Exercise and sport sciences reviews, 38*(3).

Ozcivici, E., Luu, Y. K., Adler, B., Qin, Y.-X., Rubin, J., Judex, S., & Rubin, C. T. (2010). Mechanical signals as anabolic agents in bone. *Nature Reviews Rheumatology, 6*(1), 50-59.

Padilla, F., & Laugier, P. (2005). Recent developments in trabecular bone characterization using ultrasound. *Current osteoporosis reports, 3*(2), 64-69.

Pagnotti, G. M., & Styner, M. (2016). Exercise Regulation of Marrow Adipose Tissue. *Frontiers in Endocrinology, 7*(94).

Pagnotti, G. M., Styner, M., Uzer, G., Patel, V. S., Wright, L. E., Ness, K. K., . . . Rubin, C. T. (2019). Combating osteoporosis and obesity with exercise: leveraging cell mechanosensitivity. *Nature Reviews Endocrinology, 15*(6), 339-355.

Papageorgiou, M., Elliott-Sale, K. J., Parsons, A., Tang, J. C., Greeves, J. P., Fraser, W. D., & Sale, C. (2017). Effects of reduced energy availability on bone metabolism in women and men. *Bone, 105*, 191-199.

Pearson, O. M., & Lieberman, D. E. (2004). The aging of Wolff's “law”: ontogeny and responses to mechanical loading in cortical bone. *American journal of physical anthropology, 125*(S39), 63-99.

Pedersen, B. K. (2019). Physical activity and muscle–brain crosstalk. *Nature Reviews Endocrinology, 15*(7), 383-392.

Pedersen, B. K., & Saltin, B. (2006). Evidence for prescribing exercise as therapy in chronic disease. *Scandinavian journal of medicine & science in sports, 16*(S1), 3-63.

Pedersen, B. K., & Saltin, B. (2015). Exercise as medicine–evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scandinavian journal of medicine & science in sports, 25*, 1-72.

Pedersen, M. T., Randers, M. B., Skotte, J. H., & Krustrup, P. (2009). Recreational soccer can improve the reflex response to sudden trunk loading among untrained women. *The Journal of Strength & Conditioning Research, 23*(9), 2621-2626.

Pellikaan, P., Giarmatzis, G., Vander Sloten, J., Verschueren, S., & Jonkers, I. (2018). Ranking of osteogenic potential of physical exercises in postmenopausal women based on femoral neck strains. *PLoS One, 13*(4), e0195463.

Pérez, L. M., Pareja-Galeano, H., Sanchis-Gomar, F., Emanuele, E., Lucia, A., & Gálvez, B. G. (2016). ‘Adipaging’: ageing and obesity share biological hallmarks related to a dysfunctional adipose tissue. *The Journal of physiology, 594*(12), 3187-3207.

Petersen, C., Pyne, D., Portus, M., & Dawson, B. (2009). Validity and reliability of GPS units to monitor cricket-specific movement patterns. *International journal of sports physiology and performance, 4*(3), 381-393.

Pinho, S., & Frenette, P. S. (2019). Haematopoietic stem cell activity and interactions with the niche. *Nature Reviews Molecular Cell Biology, 20*(5), 303-320.

Pivonka, P., Park, A., & Forwood, M. R. (2018). Functional adaptation of bone: the mechanostat and beyond. In *Multiscale mechanobiology of bone remodeling and adaptation* (pp. 1-60): Springer.

Plaza-Carmona, M., Vicente-Rodríguez, G., Gómez-Cabello, A., Martín-García, M., Sánchez-Sánchez, J., Gallardo, L., & Ara, I. (2016). Higher bone mass in prepubertal and peripubertal female footballers. *European journal of sport science, 16*(7), 877-883.

Popp, K. L., Turkington, V., Hughes, J. M., Xu, C., Unnikrishnan, G., Reifman, J., & Bouxsein, M. L. (2019). Skeletal loading score is associated with bone microarchitecture in young adults. *Bone, 127*, 360-366.

Portas, M. D., Harley, J. A., Barnes, C. A., & Rush, C. J. (2010). The validity and reliability of 1-Hz and 5-Hz global positioning systems for linear, multidirectional, and soccer-specific activities. *International journal of sports physiology and performance, 5*(4), 448-458.

Póvoas, S. C. A., Castagna, C., Resende, C., Coelho, E. F., Silva, P., Santos, R., . . . Krustrup, P. (2017). Physical and Physiological Demands of Recreational Team Handball for Adult Untrained Men. *BioMed Research International, 2017*, 6204603.

Prawiradilaga, R. S., Madsen, A. O., Jørgensen, N. R., & Helge, E. W. (2020). Acute response of biochemical bone turnover markers and the associated ground reaction forces to high-impact exercise in postmenopausal women. *Biology of Sport, 37*(1), 41.

Puddle, D. L., & Maulder, P. S. (2013). Ground reaction forces and loading rates associated with parkour and traditional drop landing techniques. *J Sports Sci Med, 12*(1), 122-129.

Qi, Z., Liu, W., & Lu, J. (2016). The mechanisms underlying the beneficial effects of exercise on bone remodeling: roles of bone-derived cytokines and microRNAs. *Progress in Biophysics and Molecular biology, 122*(2), 131-139.

Quagliarella, L., Sasanelli, N., Belgiovine, G., Moretti, L., & Moretti, B. (2010). Evaluation of Standing Vertical Jump by Ankles Acceleration Measurement. *The Journal of Strength & Conditioning Research, 24*(5).

Raggatt, L. J., & Partridge, N. C. (2010). Cellular and molecular mechanisms of bone remodeling. *Journal of Biological Chemistry, 285*(33), 25103-25108.

Rahnama, N., Reilly, T., Lees, A., & Graham-Smith, P. (2003). Muscle fatigue induced by exercise simulating the work rate of competitive soccer. *Journal of Sports Sciences, 21*(11), 933-942.

Rampinini, E., Coutts, A. J., Castagna, C., Sassi, R., & Impellizzeri, F. (2007). Variation in top level soccer match performance. *International journal of sports medicine, 28*(12), 1018-1024.

Randers, M. B., Nybo, L., Petersen, J., Nielsen, J. J., Christiansen, L., Bendiksen, M., . . . Krustrup, P. (2010). Activity profile and physiological response to football training for untrained males and females, elderly and youngsters: influence of the number of players. *Scandinavian Journal of Medicine & Science in Sports, 20*, 14-23.

Rantalainen, T., Hoffren, M., Linnamo, V., Heinonen, A., Komi, P., Avela, J., & Nindl, B. (2011). Three-month bilateral hopping intervention is ineffective in initiating bone biomarker response in healthy elderly men. *European journal of applied physiology, 111*(9), 2155-2162.

Rantalainen, T., Sievänen, H., Linnamo, V., Hoffrén, M., Ishikawa, M., Kyröläinen, H., . . . Heinonen, A. (2009). Bone rigidity to neuromuscular performance ratio in young and elderly men. *Bone, 45*(5), 956-963.

Rauch, F., Sievanen, H., Boonen, S., Cardinale, M., Degens, H., Felsenberg, D., . . . Rittweger, J. (2010). Reporting whole-body vibration intervention studies: recommendations of the International Society of Musculoskeletal and Neuronal Interactions. *Journal of musculoskeletal & neuronal interactions, 10*.

Rauchenzauner, M., Schmid, A., Heinz-Erian, P., Kapelari, K., Falkensammer, G., Griesmacher, A., . . . Hogler, W. (2007). Sex-and age-specific reference curves for serum markers of bone turnover in healthy children from 2 months to 18 years. *The Journal of Clinical Endocrinology & Metabolism, 92*(2), 443-449.

Rebelo, A., Brito, J., Seabra, A., Oliveira, J., Drust, B., & Krustrup, P. (2012). A new tool to measure training load in soccer training and match play. *International Journal of Sports Medicine, 33*(04), 297-304.

Rebelo, A. N. C., Silva, P., Rago, V., Barreira, D., & Krustrup, P. (2016). Differences in strength and speed demands between 4v4 and 8v8 small-sided football games. *Journal of Sports Sciences, 34*(24), 2246-2254.

Reichert, F. F., Barros, A. J. D., Domingues, M. R., & Hallal, P. C. (2007). The Role of Perceived Personal Barriers to Engagement in Leisure-Time Physical Activity. *American Journal of Public Health, 97*(3), 515-519.

Reid, I. R., Bolland, M. J., & Grey, A. (2014). Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *The Lancet, 383*(9912), 146-155.

Reznikov, N., Bilton, M., Lari, L., Stevens, M. M., & Kröger, R. (2018). Fractal-like hierarchical organization of bone begins at the nanoscale. *Science, 360*(6388).

Reznikov, N., Shahar, R., & Weiner, S. (2014). Bone hierarchical structure in three dimensions. *Acta biomaterialia, 10*(9), 3815-3826.

Rho, J.-Y., Kuhn-Spearing, L., & Zioupos, P. (1998). Mechanical properties and the hierarchical structure of bone. *Medical engineering & physics, 20*(2), 92-102.

Rhodes, R. E., Janssen, I., Bredin, S. S. D., Warburton, D. E. R., & Bauman, A. (2017). Physical activity: Health impact, prevalence, correlates and interventions. *Psychology & Health, 32*(8), 942-975.

Rittweger, J. (2010). Vibration as an exercise modality: how it may work, and what its potential might be. *European journal of applied physiology, 108*(5), 877-904.

Robling, A. G., Burr, D. B., & Turner, C. H. (2001). Recovery periods restore mechanosensitivity to dynamically loaded bone. *Journal of Experimental Biology, 204*(19), 3389-3399.

Robling, A. G., Castillo, A. B., & Turner, C. H. (2006). Biomechanical and molecular regulation of bone remodeling. *Annu. Rev. Biomed. Eng., 8*, 455-498.

Robling, A. G., Hinant, F. M., Burr, D. B., & Turner, C. H. (2002). Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *Journal of Bone and Mineral Research, 17*(8), 1545-1554.

Rodacki, A. L. F., Fowler, N. E., & Bennett, S. J. (2002). Vertical jump coordination: fatigue effects. *Medicine & Science in Sports & Exercise, 34*(1), 105-116.

Rogers, R. S., Dawson, A. W., Wang, Z., Thyfault, J. P., & Hinton, P. S. (2011). Acute response of plasma markers of bone turnover to a single bout of resistance training or plyometrics. *J Appl Physiol (1985), 111*(5), 1353-1360.

Roloff, Z. A., Dicks, N. D., Krynski, L. M., Hartman, M. E., Ekkekakis, P., & Pettitt, R. W. (2020). Ratings of affective valence closely track changes in oxygen uptake: Application to high-intensity interval exercise. *Performance Enhancement & Health, 7*(3), 100158.

Roubenoff, R., Kehayias, J. J., Dawson-Hughes, B., & Heymsfield, S. B. (1993). Use of dual-energy x-ray absorptiometry in body-composition studies: not yet a “gold standard”. *The American journal of clinical nutrition, 58*(5), 589-591.

Rowlands, A. V., Fairclough, S. J., Yates, T., Edwardson, C. L., Davies, M., Munir, F., . . . Stiles, V. H. (2019). Activity Intensity, Volume, and Norms: Utility and Interpretation of Accelerometer Metrics. *Med Sci Sports Exerc, 51*(11), 2410-2422.

Rowlands, A. V., & Stiles, V. H. (2012). Accelerometer counts and raw acceleration output in relation to mechanical loading. *Journal of Biomechanics, 45*(3), 448-454.

Rubin, C. T. (1984). Skeletal strain and the functional significance of bone architecture. *Calcified tissue international, 36*(1), S11-S18.

Rubin, C. T., & Lanyon, L. E. (1985). Regulation of bone mass by mechanical strain magnitude. *Calcified tissue international, 37*(4), 411-417.

Ruegsegger, G. N., & Booth, F. W. (2018). Health benefits of exercise. *Cold Spring Harbor perspectives in medicine, 8*(7), a029694.

Santos-Rocha, R. A., Oliveira, C. S., & Veloso, A. P. (2006). Osteogenic index of step exercise depending on choreographic movements, session duration, and stepping rate. *British journal of sports medicine, 40*(10), 860-866.

Sarmento, H., Manuel Clemente, F., Marques, A., Milanovic, Z., David Harper, L., & Figueiredo, A. (2020). Recreational football is medicine against non-communicable diseases: A systematic review. *Scandinavian Journal of Medicine & Science in Sports, 30*(4), 618-637.

Schantz, P., & Åstrand, P.-O. (1984). Physiological characteristics of classical ballet. *Medicine and science in sports and exercise, 16*(5), 472-476.

Schoenau, E. (2005). From mechanostat theory to development of the" Functional Muscle-Bone-Unit". *Journal of Musculoskeletal and Neuronal Interactions, 5*(3), 232.

Schraders, K., Zatta, G., Kruger, M., Coad, J., Weber, J., Brough, L., & Thomson, J. (2019). Quantitative Ultrasound and Dual X-Ray Absorptiometry as Indicators of Bone Mineral Density in Young Women and Nutritional Factors Affecting It. *Nutrients, 11*(10), 2336.

Schutz, Y., & Herren, R. (2000). Assessment of speed of human locomotion using a differential satellite global positioning system. *Medicine and science in sports and exercise, 32*(3), 642-646.

Sebring, N. G., Denkinger, B. I., Menzie, C. M., Yanoff, L. B., Parikh, S. J., & Yanovski, J. A. (2007). Validation of three food frequency questionnaires to assess dietary calcium intake in adults. *Journal of the American Dietetic Association, 107*(5), 752-759.

Setuain, I., Martinikorena, J., Gonzalez-Izal, M., Martinez-Ramirez, A., Gómez, M., Alfaro-Adrián, J., & Izquierdo, M. (2016). Vertical jumping biomechanical evaluation through the use of an inertial sensor-based technology. *Journal of Sports Sciences, 34*(9), 843-851.

Shen, W., Chen, J., Gantz, M., Punyanitya, M., Heymsfield, S. B., Gallagher, D., . . . Gilsanz, V. (2012). MRI-measured pelvic bone marrow adipose tissue is inversely related to DXA-measured bone mineral in younger and older adults. *European Journal of Clinical Nutrition, 66*(9), 983-988.

Shen, W., Chen, J., Gantz, M., Velasquez, G., Punyanitya, M., & Heymsfield, S. B. (2012). A single MRI slice does not accurately predict visceral and subcutaneous adipose tissue changes during weight loss. *Obesity, 20*(12), 2458-2463.

Silva, A., Heymsfield, S., & Sardinha, L. (2013). Assessing body composition in taller or broader individuals using dual-energy X-ray absorptiometry: a systematic review. *European journal of clinical nutrition, 67*(10), 1012-1021.

SIPILÄ, S., TAAFFE, D. R., CHENG, S., PUOLAKKA, J., TOIVANEN, J., & SUOMINEN, H. (2001). Effects of hormone replacement therapy and high-impact physical exercise on skeletal muscle in post-menopausal

women: a randomized placebo-controlled study. *Clinical science, 101*(2), 147-157.

Sirola, J., & Rikkonen, T. (2005). Muscle performance after the menopause. *British Menopause Society Journal, 11*(2), 45-50.

Skoradal, M. B., Helge, E. W., Jørgensen, N. R., Mortensen, J., Weihe, P., Krustrup, P., & Mohr, M. (2018). Osteogenic impact of football training in 55-to 70-year-old women and men with prediabetes. *Scandinavian journal of medicine & science in sports, 28*, 52-60.

Slatkovska, L., Alibhai, S., Beyene, J., & Cheung, A. (2010). Effect of whole-body vibration on BMD: a systematic review and meta-analysis. *Osteoporosis international, 21*(12), 1969-1980.

Söderman, K., Bergström, E., Lorentzon, R., & Alfredson, H. (2000). Bone mass and muscle strength in young female soccer players. *Calcified tissue international, 67*(4), 297-303.

Solmi, M., Veronese, N., Favaro, A., Santonastaso, P., Manzato, E., Sergi, G., & Correll, C. U. (2015). Inflammatory cytokines and anorexia nervosa: A meta-analysis of cross-sectional and longitudinal studies. *Psychoneuroendocrinology, 51*, 237-252.

Song, L. (2017). Calcium and bone metabolism indices. In *Advances in clinical chemistry* (Vol. 82, pp. 1-46): Elsevier.

Sornay-Rendu, E., Boutroy, S., Vilayphiou, N., Claustrat, B., & Chapurlat, R. D. (2013). In Obese Postmenopausal Women, Bone Microarchitecture and Strength Are Not Commensurate to Greater Body Weight: The Os des Femmes de Lyon (OFELY) Study. *Journal of Bone and Mineral Research, 28*(7), 1679-1687.

Srinivasan, S., Weimer, D. A., Agans, S. C., Bain, S. D., & Gross, T. S. (2002). Low-Magnitude Mechanical Loading Becomes Osteogenic When Rest Is Inserted Between Each Load Cycle. *Journal of Bone and Mineral Research, 17*(9), 1613-1620.

Staal, S., Sjodin, A., Fahrenholtz, I., Bonnesen, K., & Melin, A. K. (2018). Low RMRratio as a Surrogate Marker for Energy Deficiency, the Choice of Predictive Equation Vital for Correctly Identifying Male and Female Ballet Dancers at Risk. *Int J Sport Nutr Exerc Metab, 28*(4), 412-418.

STAND, P. (2007). The female athlete triad. *Med. Sci. Sports Exerc, 39*(10), 1867-1882.

Standing, R. J., & Maulder, P. S. (2015). A Comparison of the Habitual Landing Strategies from Differing Drop Heights of Parkour Practitioners (Traceurs) and Recreationally Trained Individuals. *J Sports Sci Med, 14*(4), 723-731.

Stevens, T. G., de Ruiter, C. J., van Niel, C., van de Rhee, R., Beek, P. J., & Savelsbergh, G. J. (2014). Measuring acceleration and deceleration in soccer-specific movements using a local position measurement (LPM)

system. *International journal of sports physiology and performance, 9*(3), 446-456.

Stiles, V. H., Metcalf, B. S., Knapp, K. M., & Rowlands, A. V. (2017). A small amount of precisely measured high-intensity habitual physical activity predicts bone health in pre-and post-menopausal women in UK Biobank. *International Journal of Epidemiology, 46*(6), 1847-1856.

Stojanović, E., Stojiljković, N., Scanlan, A. T., Dalbo, V. J., Berkelmans, D. M., & Milanović, Z. (2018). The Activity Demands and Physiological Responses Encountered During Basketball Match-Play: A Systematic Review. *Sports Medicine, 48*(1), 111-135.

Sweeting, A. J., Cormack, S. J., Morgan, S., & Aughey, R. J. (2017). When is a sprint a sprint? A review of the analysis of team-sport athlete activity profile. *Frontiers in physiology, 8*, 432.

Szulc, P. (2018). Bone turnover: Biology and assessment tools. *Best Practice & Research Clinical Endocrinology & Metabolism, 32*(5), 725-738.

Szulc, P., Kaufman, J. M., & Delmas, P. D. (2007). Biochemical assessment of bone turnover and bone fragility in men. *Osteoporosis International, 18*(11), 1451-1461.

Szulc, P., Naylor, K., Hoyle, N., Eastell, R., & Leary, E. (2017). Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. *Osteoporosis International, 28*(9), 2541-2556.

Taaffe, D. R., Sipilä, S., Cheng, S., Puolakka, J., Toivanen, J., & Suominen, H. (2005). The effect of hormone replacement therapy and/or exercise on skeletal muscle attenuation in postmenopausal women: a yearlong intervention. *Clinical Physiology and Functional Imaging, 25*(5), 297-304.

Taylor, J. B., Wright, A. A., Dischiavi, S. L., Townsend, M. A., & Marmon, A. R. (2017). Activity demands during multi-directional team sports: a systematic review. *Sports Medicine, 47*(12), 2533-2551.

Tenforde, A. S., Barrack, M. T., Nattiv, A., & Fredericson, M. (2016). Parallels with the Female Athlete Triad in Male Athletes. *Sports Medicine, 46*(2), 171-182.

Theodoropoulos, J. S., Bettle, J., & Kosy, J. D. (2020). The use of GPS and inertial devices for player monitoring in team sports: A review of current and future applications. *Orthopedic reviews, 12*(1), 7863-7863.

Thomas, S., Reading, J., & Shephard, R. J. (1992). Revision of the physical activity readiness questionnaire (PAR-Q). *Canadian journal of sport sciences*.

Thompson, W. R., Rubin, C. T., & Rubin, J. (2012). Mechanical regulation of signaling pathways in bone. *Gene, 503*(2), 179-193.

Tobeiha, M., Moghadasian, M. H., Amin, N., & Jafarnejad, S. (2020). RANKL/RANK/OPG Pathway: A Mechanism Involved in Exercise-Induced Bone Remodeling. *BioMed Research International, 2020*, 6910312.

Tobias, J. H. (2003). At the crossroads of skeletal responses to estrogen and exercise. *Trends Endocrinol Metab, 14*(10), 441-443.

Tommasi, M., Bacciottini, L., Benucci, A., Brocchi, A., Passeri, A., Saracini, D., . . . Cappelli, G. (1996). Serum biochemical markers of bone turnover in healthy infants and children. *The International journal of biological markers, 11*(3), 159-164.

Torma, F., Gombos, Z., Jokai, M., Takeda, M., Mimura, T., & Radak, Z. (2019). High intensity interval training and molecular adaptive response of skeletal muscle. *Sports Medicine and Health Science, 1*(1), 24-32.

Torres-Costoso, A., Vlachopoulos, D., Ubago-Guisado, E., Ferri-Morales, A., Cavero-Redondo, I., Martinez-Vizcaino, V., & Gracia-Marco, L. (2018). Agreement Between Dual-Energy X-Ray Absorptiometry and Quantitative Ultrasound to Evaluate Bone Health in Adolescents: The PRO-BONE Study. *Pediatr Exerc Sci, 30*(4), 466-473.

Trimpou, P., Bosaeus, I., Bengtsson, B.-Å., & Landin-Wilhelmsen, K. (2010). High correlation between quantitative ultrasound and DXA during 7 years of follow-up. *European Journal of Radiology, 73*(2), 360-364.

Troy, K. L., Edwards, W. B., Bhatia, V. A., & Bareither, M. L. (2013). In vivo loading model to examine bone adaptation in humans: a pilot study. *Journal of orthopaedic research, 31*(9), 1406-1413.

Troy, K. L., Mancuso, M. E., Butler, T. A., & Johnson, J. E. (2018). Exercise Early and Often: Effects of Physical Activity and Exercise on Women's Bone Health. *Int J Environ Res Public Health, 15*(5).

Troy, K. L., Mancuso, M. E., Johnson, J. E., Wu, Z., Schnitzer, T. J., & Butler, T. A. (2020). Bone Adaptation in Adult Women Is Related to Loading Dose: A 12-Month Randomized Controlled Trial. *Journal of Bone and Mineral Research, 35*(7), 1300-1312.

Tschakert, G., Kroepfl, J., Mueller, A., Moser, O., Groeschl, W., & Hofmann, P. (2015). How to regulate the acute physiological response to "aerobic" high-intensity interval exercise. *Journal of sports science & medicine, 14*(1), 29-36.

Tsigilis, N., Douda, H., & Tokmakidis, S. P. (2002). Test-Retest Reliability of the Eurofit Test Battery Administered to University Students. *Perceptual and Motor Skills, 95*(3_suppl), 1295-1300.

Turner, C., Akhter, M. P., Raab, D., Kimmel, D., & Recker, R. R. (1991). A noninvasive, in vivo model for studying strain adaptive bone modeling. *Bone, 12*(2), 73-79.

Turner, C. H. (1998). Three rules for bone adaptation to mechanical stimuli. *Bone, 23*(5), 399-407.

Turner, C. H., & Burr, D. B. (1993). Basic biomechanical measurements of bone: a tutorial. *Bone, 14*(4), 595-608.

Turner, C. H., Forwood, M. R., & Otter, M. W. (1994). Mechanotransduction in bone: do bone cells act as sensors of fluid flow? *The FASEB journal, 8*(11), 875-878.

Turner, C. H., & Pavalko, F. M. (1998). Mechanotransduction and functional response of the skeleton to physical stress: The mechanisms and mechanics of bone adaptation. *Journal of Orthopaedic Science, 3*(6), 346-355.

Turner, C. H., & Robling, A. G. (2003). Designing exercise regimens to increase bone strength. *Exercise and sport sciences reviews, 31*(1), 45-50.

Twitchett, E., Nevill, A., Angioi, M., Koutedakis, Y., & Wyon, M. (2011). Development, validity, and reliability of a ballet-specific aerobic fitness test. *Journal of Dance Medicine & Science, 15*(3), 123-127.

Twitchett, E. A., Koutedakis, Y., & Wyon, M. A. (2009). Physiological fitness and professional classical ballet performance: a brief review. *The Journal of Strength & Conditioning Research, 23*(9), 2732-2740.

Tyrovola, J. B., & Odont, X. (2015). The “mechanostat theory” of frost and the OPG/Rankl/RANK system. *Journal of cellular biochemistry, 116*(12), 2724-2729.

Ubago-Guisado, E., Gómez-Cabello, A., Sánchez-Sánchez, J., García-Unanue, J., & Gallardo, L. (2015). Influence of different sports on bone mass in growing girls. *Journal of sports sciences, 33*(16), 1710-1718.

Ubago-Guisado, E., Sanchez Sanchez, J., Vila Maldonado, S., & Gallardo, L. (2019). Effects of Zumba((R)) and Aquagym on Bone Mass in Inactive Middle-Aged Women. *Medicina (Kaunas), 55*(1).

Ueda, T., Nabetani, T., & Teramoto, K. (2006). Differential perceived exertion measured using a new visual analogue scale during pedaling and running. *Journal of Physiological Anthropology, 25*(2), 171-177.

Uth, J., Hornstrup, T., Christensen, J. F., Christensen, K. B., Jørgensen, N. R., Schmidt, J. F., . . . Helge, E. W. (2016). Efficacy of recreational football on bone health, body composition, and physical functioning in men with prostate cancer undergoing androgen deprivation therapy: 32-week follow-up of the FC prostate randomised controlled trial. *Osteoporosis International, 27*(4), 1507-1518.

Vaeyens, R., Lenoir, M., Williams, A. M., & Philippaerts, R. M. (2008). Talent identification and development programmes in sport. *Sports medicine, 38*(9), 703-714.

Vainionpaa, A., Korpelainen, R., Sievanen, H., Vihriala, E., Leppaluoto, J., & Jamsa, T. (2007). Effect of impact exercise and its intensity on bone geometry at weight-bearing tibia and femur. *Bone, 40*(3), 604-611.

Vainionpää, A., Korpelainen, R., Sievänen, H., Vihriälä, E., Leppäluoto, J., & Jämsä, T. (2007). Effect of impact exercise and its intensity on bone geometry at weight-bearing tibia and femur. *Bone, 40*(3), 604-611.

Vainionpää, A., Korpelainen, R., Vihriälä, E., Rinta–Paavola, A., Leppäluoto, J., & Jämsä, T. (2006). Intensity of exercise is associated with bone density change in premenopausal women. *Osteoporosis International, 17*(3), 455-463.

Valentino, R., Savastano, S., Tommaselli, A. P., D'amore, G., Dorato, M., & Lombardi, G. (2001). The influence of intense ballet training on trabecular bone mass, hormone status, and gonadotropin structure in young women. *The Journal of Clinical Endocrinology & Metabolism, 86*(10), 4674-4678.

Van Coeverden, S., Netelenbos, J., De Ridder, C., Roos, J., Popp-Snijders, C., & Delemarre-van de Waal, H. (2002). Bone metabolism markers and bone mass in healthy pubertal boys and girls. *Clinical endocrinology, 57*(1), 107-116.

Van der Berg, J. D., Stehouwer, C. D., Bosma, H., van der Velde, J. H., Willems, P. J., Savelberg, H. H., . . . Henry, R. M. (2016). Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: The Maastricht Study. *Diabetologia, 59*(4), 709-718.

Varley, I., Hughes, D., Greeves, J., Fraser, W., & Sale, C. (2017). Increased training volume improves bone density and cortical area in adolescent football players. *International journal of sports medicine*.

Verschueren, S. M., Roelants, M., Delecluse, C., Swinnen, S., Vanderschueren, D., & Boonen, S. (2004). Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. *Journal of Bone and Mineral Research, 19*(3), 352-359.

Vicente-Rodriguez, G., Dorado, C., Ara, I., Perez-Gomez, J., Olmedillas, H., Delgado-Guerra, S., & Calbet, J. (2007). Artistic versus rhythmic gymnastics: effects on bone and muscle mass in young girls. *International journal of sports medicine*.

Vickery, W. M., Dascombe, B. J., Baker, J. D., Higham, D. G., Spratford, W. A., & Duffield, R. (2014). Accuracy and reliability of GPS devices for measurement of sports-specific movement patterns related to cricket, tennis, and field-based team sports. *The Journal of Strength & Conditioning Research, 28*(6), 1697-1705.

Vlachopoulos, D., Barker, A. R., Ubago-Guisado, E., Williams, C. A., & Gracia-Marco, L. (2018). The effect of a high-impact jumping intervention on

bone mass, bone stiffness and fitness parameters in adolescent athletes. *Archives of osteoporosis, 13*(1), 128.

Vlachopoulos, D., Barker, A. R., Ubago-Guisado, E., Fatouros, I. G., Knapp, K. M., Williams, C. A., & Gracia-Marco, L. (2017). Longitudinal Adaptations of Bone Mass, Geometry, and Metabolism in Adolescent Male Athletes: The PRO-BONE Study. *Journal of Bone and Mineral Research, 32*(11), 2269-2277.

Wajchenberg, B. L. (2000). Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocrine reviews, 21*(6), 697-738.

Walker, G. E., Marzullo, P., Prodam, F., Bona, G., & Di Blasio, A. M. (2014). Obesity modifies expression profiles of metabolic markers in superficial and deep subcutaneous abdominal adipose tissue depots. *Endocrine, 46*(1), 99-106.

Walker, I. J., Nordin-Bates, S. M., & Redding, E. (2010). Talent identification and development in dance: A review of the literature. *Research in Dance Education, 11*(3), 167-191.

Walker, I. V., Smith, C. R., Davies, J. H., Inskip, H. M., & Baird, J. (2020). Methods for determining pubertal status in research studies: literature review and opinions of experts and adolescents. *Journal of Developmental Origins of Health and Disease, 11*(2), 168-187.

Wallace, I. J., Demes, B., Mongle, C., Pearson, O. M., Polk, J. D., & Lieberman, D. E. (2014). Exercise-induced bone formation is poorly linked to local strain magnitude in the sheep tibia. *PloS one, 9*(6), e99108.

Wang, J., Thornton, J. C., Bari, S., Williamson, B., Gallagher, D., Heymsfield, S. B., . . . Mayer, L. (2003). Comparisons of waist circumferences measured at 4 sites. *The American journal of clinical nutrition, 77*(2), 379-384.

Warner, S., Shea, J., Miller, S., & Shaw, J. (2006). Adaptations in cortical and trabecular bone in response to mechanical loading with and without weight bearing. *Calcified tissue international, 79*(6), 395-403.

Warr-di Piero, D., Valverde-Esteve, T., Redondo-Castán, J. C., Pablos-Abella, C., & Sánchez-Alarcos Díaz-Pintado, J. V. (2018). Effects of work-interval duration and sport specificity on blood lactate concentration, heart rate and perceptual responses during high intensity interval training. *PLOS ONE, 13*(7), e0200690.

Warren, M. P., Brooks-Gunn, J., Fox, R. P., Holderness, C. C., Hyle, E. P., & Hamilton, W. G. (2002). Osteopenia in exercise-associated amenorrhea using ballet dancers as a model: a longitudinal study. *The Journal of Clinical Endocrinology & Metabolism, 87*(7), 3162-3168.

Warren, M. P., Brooks-Gunn, J., Fox, R. P., Holderness, C. C., Hyle, E. P., Hamilton, W. G., & Hamilton, L. (2003). Persistent osteopenia in ballet dancers with amenorrhea and delayed menarche despite hormone therapy: A longitudinal study. *Fertility and Sterility, 80*, 398-404.

Wehner, T., Claes, L., & Simon, U. (2009). Internal loads in the human tibia during gait. *Clinical Biomechanics, 24*(3), 299-302.

Weinbaum, S., Guo, P., & You, L. (2001). A new view of mechanotransduction and strain amplification in cells with microvilli and cell processes. *Biorheology, 38*(2, 3), 119-142.

Weiner, S., & Traub, W. (1992). Bone structure: from ångstroms to microns. *The FASEB journal, 6*(3), 879-885.

Weiner, S., & Wagner, H. D. (1998). The material bone: structure-mechanical function relations. *Annual review of materials science, 28*(1), 271-298.

Welch, N., McNaughton, S. A., Hunter, W., Hume, C., & Crawford, D. (2009). Is the perception of time pressure a barrier to healthy eating and physical activity among women? *Public health nutrition, 12*(7), 888-895.

Wen, H., Huang, T.-H., Li, T., Chong, P., & Ang, B. (2017). Effects of short-term step aerobics exercise on bone metabolism and functional fitness in postmenopausal women with low bone mass. *Osteoporosis International, 28*(2), 539-547.

Weston, K. S., Wisløff, U., & Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *British journal of sports medicine, 48*(16), 1227-1234.

Wewege, M. A., & Ward, R. E. (2018). Bone mineral density in pre-professional female ballet dancers: A systematic review and meta-analysis. *Journal of science and medicine in sport, 21*(8), 783-788.

WHO. (1995). *Physical status: The use of and interpretation of anthropometry, Report of a WHO Expert Committee*: World Health Organization.

Williams, N. I., Helmreich, D. L., Parfitt, D. B., Caston-Balderrama, A., & Cameron, J. L. (2001). Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. *The Journal of Clinical Endocrinology & Metabolism, 86*(11), 5184-5193.

Williams, N. I., Koltun, K. J., Strock, N. C. A., & De Souza, M. J. (2019). Female Athlete Triad and Relative Energy Deficiency in Sport: A Focus on Scientific Rigor. *Exercise and sport sciences reviews, 47*(4), 197-205.

Williams, N. I., Leidy, H. J., Hill, B. R., Lieberman, J. L., Legro, R. S., & Souza, M. J. D. (2015). Magnitude of daily energy deficit predicts frequency but not severity of menstrual disturbances associated with exercise and caloric restriction. *American Journal of Physiology-Endocrinology and Metabolism, 308*(1), E29-E39.

Willoughby, T., & Copeland, J. L. (2015). Sedentary time is not independently related to postural stability or leg strength in women 50-67 years old. *Appl Physiol Nutr Metab, 40*(11), 1123-1128.

Winter, D. A. (2009). *Biomechanics and motor control of human movement*: John Wiley & Sons.

Wolman, R., Wyon, M. A., Koutedakis, Y., Nevill, A. M., Eastell, R., & Allen, N. (2013). Vitamin D status in professional ballet dancers: winter vs. summer. *Journal of Science and Medicine in Sport, 16*(5), 388-391.

Wu, D., Isaksson, P., Ferguson, S. J., & Persson, C. (2018). Young's modulus of trabecular bone at the tissue level: A review. *Acta Biomaterialia, 78*, 1-12.

Wundersitz, D. W., Gastin, P., Robertson, S., Davey, P., & Netto, K. (2015). Validation of a trunk-mounted accelerometer to measure peak impacts during team sport movements. *International journal of sports medicine, 36*(09), 742-746.

Wundersitz, D. W. T., Netto, K. J., Aisbett, B., & Gastin, P. B. (2013). Validity of an upper-body-mounted accelerometer to measure peak vertical and resultant force during running and change-of-direction tasks. *Sports Biomechanics, 12*(4), 403-412.

Wyon, M., Redding, E., Abt, G., Head, A., & Sharp, N. C. C. (2003). Development, reliability, and validity of a multistage dance specific aerobic fitness test (DAFT). *Journal of dance medicine & science, 7*(3), 80-84.

Wyon, M. A., Abt, G., Redding, E., Head, A., & Sharp, C. N. (2004). Oxygen uptake during modern dance class, rehearsal, and performance. *The Journal of Strength & Conditioning Research, 18*(3), 646-649.

Wyon, M. A., Deighan, M. A., Nevill, A. M., Doherty, M., Morrison, S. L., Allen, N., . . . George, S. (2007). The cardiorespiratory, anthropometric, and performance characteristics of an international/national touring ballet company. *Journal of Strength and Conditioning Research, 21*(2), 389.

Wyon, M. A., Koutedakis, Y., Wolman, R., Nevill, A. M., & Allen, N. (2014). The influence of winter vitamin D supplementation on muscle function and injury occurrence in elite ballet dancers: a controlled study. *Journal of science and medicine in sport, 17*(1), 8-12.

Wyon, M. A., Twitchett, E., Angioi, M., Clarke, F., Metsios, G., & Koutedakis, Y. (2011). Time motion and video analysis of classical ballet and contemporary dance performance. *International journal of sports medicine, 32*(11), 851-855.

Xu, J., Lombardi, G., Jiao, W., & Banfi, G. (2016). Effects of exercise on bone status in female subjects, from young girls to postmenopausal women: an overview of systematic reviews and meta-analyses. *Sports Medicine, 46*(8), 1165-1182.

Xu, Y., Guo, B., Gong, J., Xu, H., & Bai, Z. (2014). The correlation between calcaneus stiffness index calculated by QUS and total body BMD assessed by DXA in Chinese children and adolescents. *Journal of bone and mineral metabolism, 32*(2), 159-166.

Yang, C.-C., & Hsu, Y.-L. (2010). A review of accelerometry-based wearable motion detectors for physical activity monitoring. *Sensors, 10*(8), 7772-7788.

Yang, P.-F., Sanno, M., Ganse, B., Koy, T., Brüggemann, G.-P., Müller, L. P., & Rittweger, J. (2014). Torsion and Antero-Posterior Bending in the In Vivo Human Tibia Loading Regimes during Walking and Running. *PLOS ONE, 9*(4), e94525.

Yu, B., Lin, C.-F., & Garrett, W. E. (2006). Lower extremity biomechanics during the landing of a stop-jump task. *Clinical Biomechanics, 21*(3), 297-305.

Yuan, Y., Chen, X., Zhang, L., Wu, J., Guo, J., Zou, D., . . . Zou, J. (2016). The roles of exercise in bone remodeling and in prevention and treatment of osteoporosis. *Progress in Biophysics and Molecular Biology, 122*(2), 122-130.

Zaidell, L. N., Pollock, R. D., James, D. C., Bowtell, J. L., Newham, D. J., Sumners, D. P., & Mileva, K. N. (2019). Lower Body Acceleration and Muscular Responses to Rotational and Vertical Whole-Body Vibration at Different Frequencies and Amplitudes. *Dose-response : a publication of International Hormesis Society, 17*(1), 1559325818819946-1559325818819946.

Zhang, L., Lv, H., Zheng, H., Li, M., Yin, P., Peng, Y., . . . Tang, P. (2015). Correlation between parameters of calcaneal quantitative ultrasound and hip structural analysis in osteoporotic fracture patients. *PloS one, 10*(12), e0145879.

Zhang, P., Peterson, M., Su, G. L., & Wang, S. C. (2015). Visceral adiposity is negatively associated with bone density and muscle attenuation. *The American journal of clinical nutrition, 101*(2), 337-343.

Zhang, S., Fu, W., & Liu, Y. (2019). Changes in Lower-Limb Biomechanics, Soft Tissue Vibrations, and Muscle Activation During Unanticipated Bipedal Landings. *J Hum Kinet, 67*, 25-35.

Zhao, R., Xu, Z., & Zhao, M. (2015). Effects of Oestrogen Treatment on Skeletal Response to Exercise in the Hips and Spine in Postmenopausal Women: A Meta-Analysis. *Sports Medicine, 45*(8), 1163-1173.

Zhao, R., Zhang, M., & Zhang, Q. (2017). The effectiveness of combined exercise interventions for preventing postmenopausal bone loss: a systematic review and meta-analysis. *journal of orthopaedic & sports physical therapy, 47*(4), 241-251.

Zhao, R., Zhao, M., & Zhang, L. (2014). Efficiency of jumping exercise in improving bone mineral density among premenopausal women: a meta-analysis. *Sports medicine, 44*(10), 1393-1402.

Zouhal, H., Hammami, A., Tijani, J. M., Jayavel, A., de Sousa, M., Krustrup, P., . . . Abderrahman, A. B. (2020). Effects of Small-Sided Soccer Games on Physical Fitness, Physiological Responses, and Health Indices in

Untrained Individuals and Clinical Populations: A Systematic Review. *Sports Medicine*, 1-21.

Zürcher, S. J., Borter, N., Kränzlin, M., Neyer, P., Meyer, U., Rizzoli, R., & Kriemler, S. (2020). Relationship between bone mineral content and bone turnover markers, sex hormones and calciotropic hormones in pre- and early pubertal children. *Osteoporosis International, 31*(2), 335-349.

Web references

1. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

2. http://www.i18nguy.com/l10n/shoes.html Access date: 01.06.2013

3. https://www.bones.nih.gov/health-info/bone/bone-health/nutrition/calcium-and-vitamin-d-important-every-age

Appendix 1. Assessment tools

A1.1 Bone Questionnaire

Please complete **all** the appropriate sections, using the tick boxes where provided.

Date questionnaire completed ……

Surname …..…….Forename(s)……Title..……

Address ……

……Postcode…...……...

Telephone Number (including area code) ……

Date of Birth …… (day/month/year)

Gender Male ☐ Female ☐

Ethnic Background White [ ] Oriental [ ]

Black [ ] Mixed [ ]

Asian [ ] Other [ ]

Height …….. Weight ……..…….

GP Name ……

GP Address …….

……

GP Telephone Number……..…….

Medical History

1. Have you ever suffered from any of these conditions?

	No	Yes	Please state when diagnosed and duration of disease
Rheumatoid arthritis			
Osteoarthritis			
Ankylosing spondylitis			
Diabetes			
Overactive thyroid			
Underactive thyroid			
Cancer			
Pagets disease of bone			
Liver disease			
Kidney disease			
Gastric surgery			
Lactose intolerance (milk allergy)			
Crohn's disease			
Coeliac disease			
Irritable bowel syndrome			
Malabsorption syndrome			
Osteomalacia (rickets)			
Bulimia			
Anorexia nervosa			
Breast cancer			

2. Do you suffer from any other on-going disease? Yes [ ] No [ ]

If yes, please state disease and duration……..……

……..

3. Have any of your family (parents / brothers / sisters / children / aunts / uncles / nieces / nephews / grandparents) suffered from the following conditions?

Broken hip, spine &/or wrist? Yes [ ] No [ ] Which relative?…….....…

Other broken bones? Yes [ ] No [ ] Which relative?……..

Osteoporosis? Yes [ ] No [ ] Which relative?…….……..

4. Do any other diseases run in your family? Yes [ ] No [ ]

If yes, please state the disease, and the relatives affected..……...

…….....……

5. Have you been immobilised for more than 6 wks (complete bed rest/.

hospitalisation)? Yes [ ] No [ ]

6. Have you ever taken any of the following drugs?

Drug	No	Yes	For how long did you take them?
Corticosteroids **(Please state dose)**			
Anticonvulsants			
Diuretics			
Chemotherapy			
Immunosuppresive agents			
Heparin			
Thyroxine			
Didronel (Etidronate)			
Fosamax (Alendtronate)			
Calcitonin			
Actonel (Risidronate)			
Teriparatide (PTH)			
Protelos (Strontium Ranalate)			
Pamidronate (infusions)			
Zolendronate (injection)			
Ibandronate			
Fluoride			

7. Have you taken any other drugs fro greater than 6 months? Yes [ ] No [ ]

What drug?	For how long?

8. Do you take any of the following dietary supplements?

	No	Yes	For how long?
Multivitamins			
Calcium			
Vitamin D			
Other (please state)			

9. Have you ever fractured (broken) any bones? Yes [ ] No [ ]

If yes, please state how old you were, which bone(s) you broke, and how it happened, (please be as accurate and specific as possible):

Age	Bone	What Happened?

10. Do you, or have you in the past suffered from back pain? Yes [ ] No [ ]

If yes, how many episodes and how severe was the pain?.....

……

11. Have you had any falls in the last year? Yes [ ] No [ ]

If yes, now many and how did they happen?

Fall No	How did it happen	Did you sustain any injuries?

Lifestyle

12. Please tick which best applies to you

Current smoker	[ ]
Ex-smoker	[ ]
Never Smoked	[ ]

If ex-smoker, what age were you when you stopped? .....

How many cigarettes did you or do you smoke per day?.....……

How many years did you or have you smoked for?……

13. How much alcohol do you drink per week?

(1 unit = ½ pint beer, a measure of spirits or a glass of wine)

Never	[ ]	11-15 units per week	[ ]
Social occasions only	[ ]	16-20 units per week	[ ]
1-5 units per week	[ ]	More than 20 units per week	[ ]
6-10 units per week	[ ]		

14. Are you vegetarian? Yes [ ] No [ ] If yes, for how long?.....…years

Are you vegan? Yes [ ] No [ ] If yes, for how long?.....…years

15. How many cups or cans of caffeine-containing beverages (coffee, tea and soft drinks such as cola) do you drink per day?

None	[ ]	11 – 15 cups/cans per day	[ ]
1 – 5 cups/cans per day	[ ]	More than 15 cups/cans per day	[ ]
6-10 cups/cans per day	[ ]		

16. How much time do you typically spend taking exercise (for example walking or cycling out of doors) each day?

None	[ ]
Some, but less than half an hour	[ ]
Half to one hour	[ ]
More than one hour	[ ]

17. Please outline any sporting or other activities you do partake in, and for how much time each week you spend doing these.

……

……

……

The rest of the questionnaire is for completion by women only

18. How old were you when your periods started?……..
Has there been any time when your periods have stopped for a time of more than 6 months except during pregnancy and menopause? Y[ ] N [ ]
If Yes, for how long did they stop?……

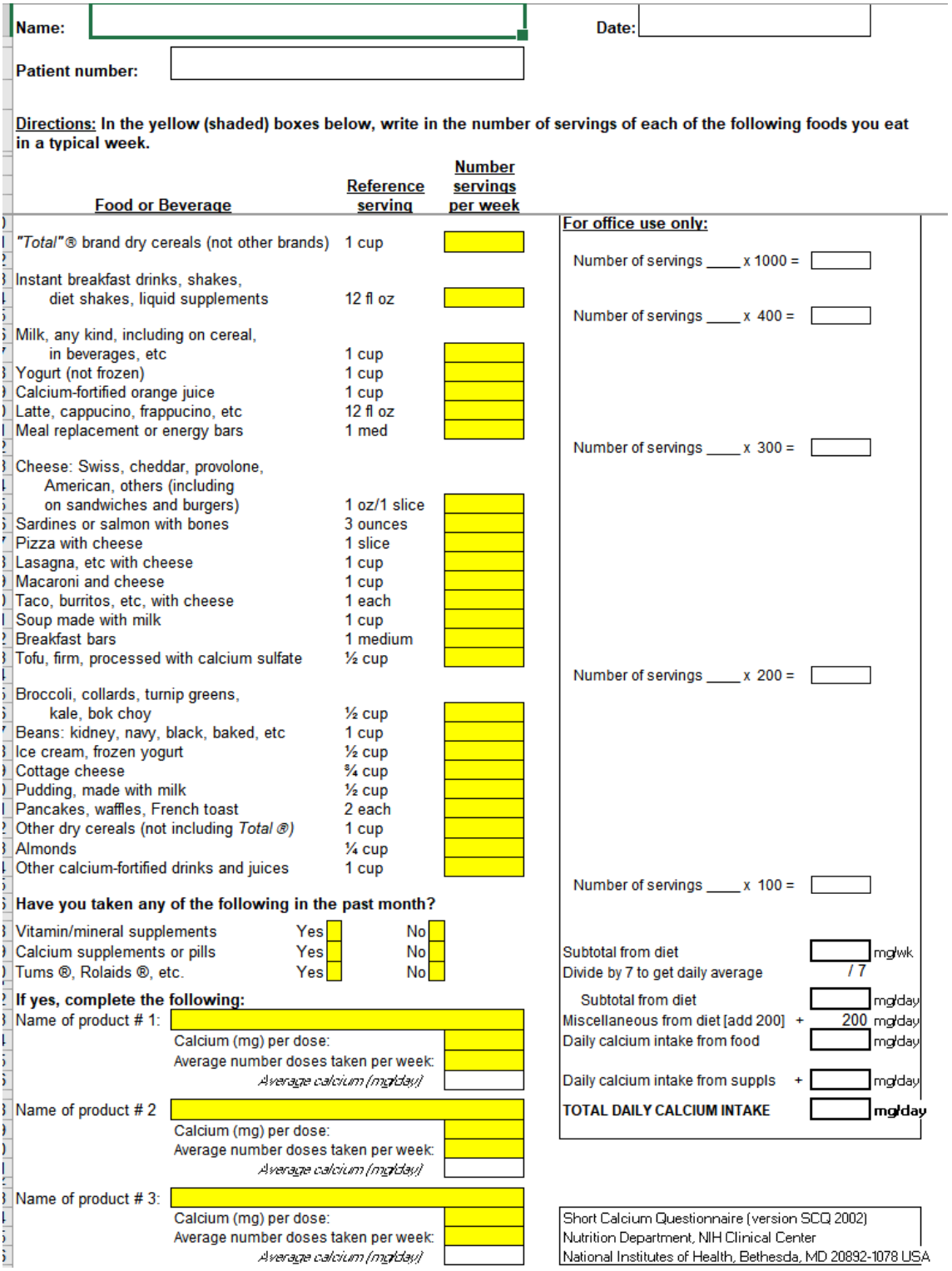
19. Have you had a hysterectomy? Yes [ ] No [ ]
If yes, at what age and for what reason? Age ……..
Reason……..

Have you had your ovaries removed? Yes [ ] No [ ] Don't know [ ]
If yes, was 1 ovary removed [ ] or both removed [ ] How old were you?

20. Are you still having natural periods? Yes [ ] No [ ]
If yes, are they regular? Yes [ ] No [ ]
If no and your periods stopped naturally, at what age did they stop?……..

21. Are you on, or have you ever taken the oral contraceptive pill? Y [ ] N [ ]
If yes, for how long have you taken it?…….
Are you still taking it? Yes [ ] No [ ]

A1.2 Calcium Food Frequency



Name: _____ **Date:** _____

Patient number: _____

Directions: **In the yellow (shaded) boxes below, write in the number of servings of each of the following foods you eat in a typical week.**

Food or Beverage	Reference serving	Number servings per week
"Total"® brand dry cereals (not other brands)	1 cup	
Instant breakfast drinks, shakes, diet shakes, liquid supplements	12 fl oz	
Milk, any kind, including on cereal, in beverages, etc	1 cup	
Yogurt (not frozen)	1 cup	
Calcium-fortified orange juice	1 cup	
Latte, cappucino, frappucino, etc	12 fl oz	
Meal replacement or energy bars	1 med	
Cheese: Swiss, cheddar, provolone, American, others (including on sandwiches and burgers)	1 oz/1 slice	
Sardines or salmon with bones	3 ounces	
Pizza with cheese	1 slice	
Lasagna, etc with cheese	1 cup	
Macaroni and cheese	1 cup	
Taco, burritos, etc, with cheese	1 each	
Soup made with milk	1 cup	
Breakfast bars	1 medium	
Tofu, firm, processed with calcium sulfate	½ cup	
Broccoli, collards, turnip greens, kale, bok choy	½ cup	
Beans: kidney, navy, black, baked, etc	1 cup	
Ice cream, frozen yogurt	½ cup	
Cottage cheese	¾ cup	
Pudding, made with milk	½ cup	
Pancakes, waffles, French toast	2 each	
Other dry cereals (not including *Total* ®)	1 cup	
Almonds	¼ cup	
Other calcium-fortified drinks and juices	1 cup	

Have you taken any of the following in the past month?

Vitamin/mineral supplements	Yes	No
Calcium supplements or pills	Yes	No
Tums ®, Rolaids ®, etc.	Yes	No

If yes, complete the following:

Name of product # 1: _____
Calcium (mg) per dose:
Average number doses taken per week:
Average calcium (mg/day)

Name of product # 2 _____
Calcium (mg) per dose:
Average number doses taken per week:
Average calcium (mg/day)

Name of product # 3: _____
Calcium (mg) per dose:
Average number doses taken per week:
Average calcium (mg/day)

For office use only:

Number of servings ____ x 1000 = ____

Number of servings ____ x 400 = ____

Number of servings ____ x 300 = ____

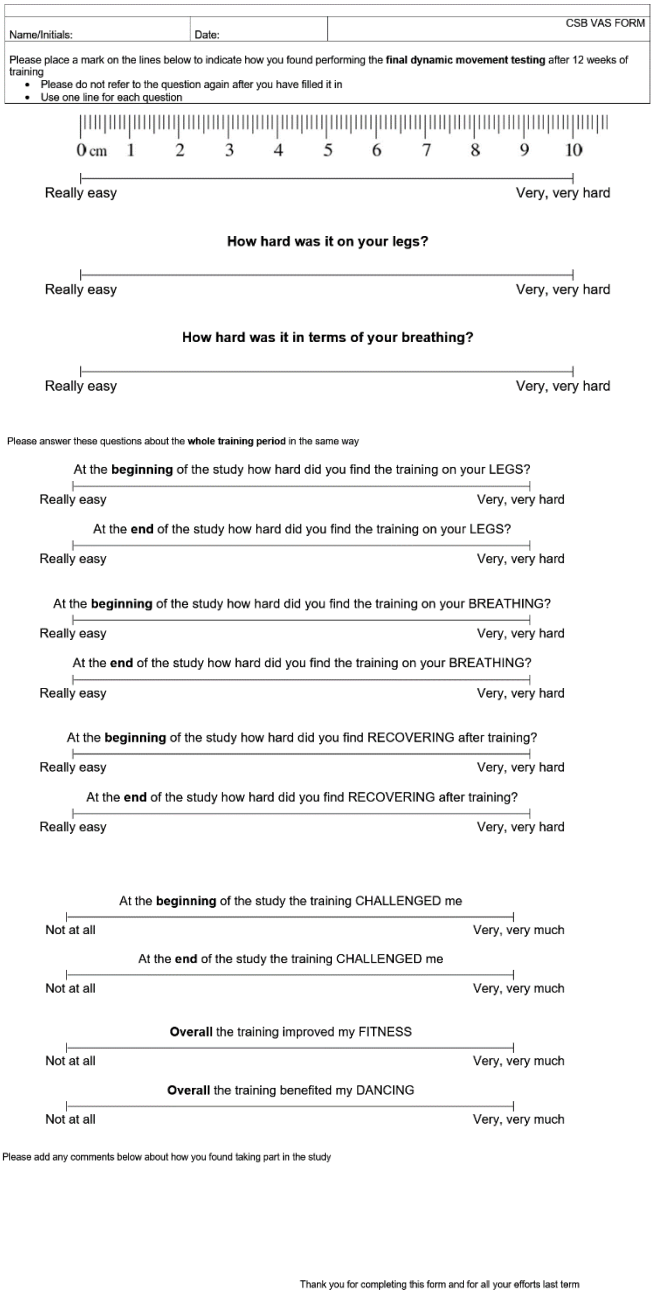
Number of servings ____ x 200 = ____

Number of servings ____ x 100 = ____

Subtotal from diet		mg/wk
Divide by 7 to get daily average	/ 7	
Subtotal from diet		mg/day
Miscellaneous from diet [add 200]	+ 200	mg/day
Daily calcium intake from food		mg/day
Daily calcium intake from suppls	+	mg/day
TOTAL DAILY CALCIUM INTAKE		**mg/day**

Short Calcium Questionnaire (version SCQ 2002)
Nutrition Department, NIH Clinical Center
National Institutes of Health, Bethesda, MD 20892-1078 USA

A1.3 Visual Analogue Scale for Dancers (Non-Validated)



CSB VAS FORM

Name/Initials: Date:

Please place a mark on the lines below to indicate how you found performing the **final dynamic movement testing** after 12 weeks of training

- Please do not refer to the question again after you have filled it in
- Use one line for each question

0 cm 1 2 3 4 5 6 7 8 9 10

Really easy — Very, very hard

How hard was it on your legs?

Really easy — Very, very hard

How hard was it in terms of your breathing?

Really easy — Very, very hard

Please answer these questions about the **whole training period** in the same way

At the **beginning** of the study how hard did you find the training on your LEGS?

Really easy — Very, very hard

At the **end** of the study how hard did you find the training on your LEGS?

Really easy — Very, very hard

At the **beginning** of the study how hard did you find the training on your BREATHING?

Really easy — Very, very hard

At the **end** of the study how hard did you find the training on your BREATHING?

Really easy — Very, very hard

At the **beginning** of the study how hard did you find RECOVERING after training?

Really easy — Very, very hard

At the **end** of the study how hard did you find RECOVERING after training?

Really easy — Very, very hard

At the **beginning** of the study the training CHALLENGED me

Not at all — Very, very much

At the **end** of the study the training CHALLENGED me

Not at all — Very, very much

Overall the training improved my FITNESS

Not at all — Very, very much

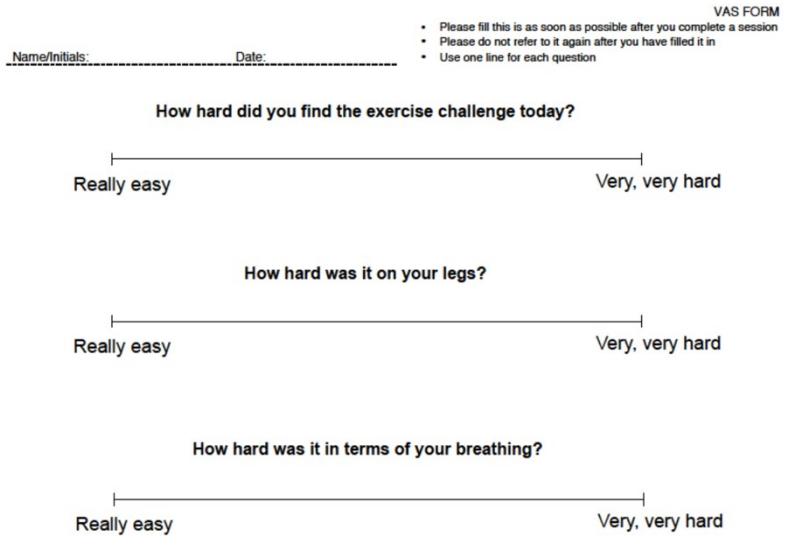
Overall the training benefited my DANCING

Not at all — Very, very much

Please add any comments below about how you found taking part in the study

Thank you for completing this form and for all your efforts last term

A1.4 Visual Analogue Scale (VAS)



VAS FORM

- Please fill this is as soon as possible after you complete a session
- Please do not refer to it again after you have filled it in
- Use one line for each question

Name/Initials: _____ Date: _____

How hard did you find the exercise challenge today?

Really easy — Very, very hard

How hard was it on your legs?

Really easy — Very, very hard

How hard was it in terms of your breathing?

Really easy — Very, very hard

A1.5 International Physical Activity Questionnaire

Long form (English) downloaded at: http://www.ipaq.ki.se

A1.6 Physical Activity Readiness Questionnaire (PAR-Q)

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

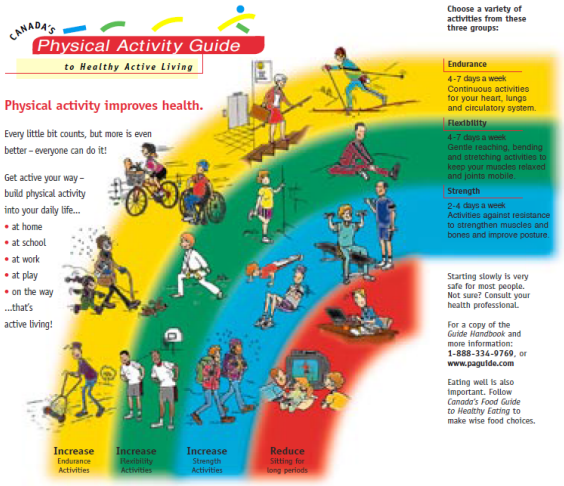
Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

continued on other side...

...continued from other side

PAR-Q & YOU

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)



Get Active Your Way, Every Day – For Life!

Scientists say accumulate 60 minutes of physical activity every day to stay healthy or improve your health. As you progress to moderate activities you can cut down to 30 minutes, 4 days a week. Add-up your activities in periods of at least 10 minutes each. Start slowly... and build up.

Time needed depends on effort

Very Light Effort	Light Effort 60 minutes+	Moderate Effort 30-60 minutes+	Vigorous Effort 20-30 minutes+	Maximum Effort
• Strolling • Dusting	• Light walking • Volleyball • Easy gardening • Stretching	• Brisk walking • Biking • Raking leaves • Swimming • Dancing • Water aerobics	• Aerobics • Jogging • Hockey • Basketball • Fast swimming • Fast dancing	• Sprinting • Racing

Range needed to stay healthy

You Can Do It – Getting started is easier than you think

Physical activity doesn t have to be very hard. Build physical activities into your daily routine.

- Walk whenever you can – get off the bus early, use the stairs instead of the elevator.
- Reduce inactivity for long periods, like watching TV.
- Get up from the couch and stretch and bend for a few minutes every hour.
- Play actively with your kids.
- Choose to walk, wheel or cycle for short trips.
- Start with a 10 minute walk – gradually increase the time.
- Find out about walking and cycling paths nearby and use them.
- Observe a physical activity class to see if you want to try it.
- Try one class to start – you don t have to make a long-term commitment.
- Do the activities you are doing now, more often.

Benefits of regular activity:	Health risks of inactivity:
• better health • improved fitness • better posture and balance • better self-esteem • weight control • stronger muscles and bones • feeling more energetic • relaxation and reduced stress • continued independent living in later life	• premature death • heart disease • obesity • high blood pressure • adult-onset diabetes • osteoporosis • stroke • depression • colon cancer

Source: Canada's Physical Activity Guide to Healthy Active Living, Health Canada, 1998 http://www.hc-sc.gc.ca/hppb/paguide/pdf/guideEng.pdf

FITNESS AND HEALTH PROFESSIONALS MAY BE INTERESTED IN THE INFORMATION BELOW:

The following companion forms are available for doctors' use by contacting the Canadian Society for Exercise Physiology (address below):

The **Physical Activity Readiness Medical Examination (PARmed-X)** – to be used by doctors with people who answer YES to one or more questions on the PAR-Q.

The **Physical Activity Readiness Medical Examination for Pregnancy (PARmed-X for Pregnancy)** – to be used by doctors with pregnant patients who wish to become more active.

References:

Arraix, G.A., Wigle, D.T., Mao, Y. (1992). Risk Assessment of Physical Activity and Physical Fitness in the Canada Health Survey Follow-Up Study. **J. Clin. Epidemiol.** 45:4 419-428.

Mottola, M., Wolfe, L.A. (1994). Active Living and Pregnancy. In: A. Quinney, L. Gauvin, T. Wall (eds.), **Toward Active Living: Proceedings of the International Conference on Physical Activity, Fitness and Health**. Champaign, IL: Human Kinetics.

PAR-Q Validation Report, British Columbia Ministry of Health, 1978.

Thomas, S., Reading, J., Shephard, R.J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). **Can. J. Spt. Sci.** 17:4 338-345.

For more information, please contact the:

Canadian Society for Exercise Physiology
202-185 Somerset Street West
Ottawa, ON K2P 0J2
Tel. 1-877-651-3755 • FAX (613) 234-3565
Online: www.csep.ca

The original PAR-Q was developed by the British Columbia Ministry of Health. It has been revised by an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Gledhill (2002).

Disponible en français sous le titre «Questionnaire sur l'aptitude à l'activité physique - Q-AAP (revisé 2002)».

Appendix 2. Ethics forms

A2.1 Approval of Ethics: Dance Grid Feasibility Study

To: Knapp, Karen
Subject: Your application for ethical approval (2013/690) has been accepted

Ethical Approval system

Your application (2013/690) entitled Feasibility of applying a dance-based Football Specific Movement simulation for 35-55 year old women (Dance Grid Feasibility Study- 'DGFS'). has been accepted
Please visit http://www.exeter.ac.uk/staff/ethicalapproval/

A2.2 Application for Ethical Approval: Acute Diverse HIIT

UNIVERSITY OF EXETER

St. Luke's Campus

14th June 2016

Dear Professor Hillsdon

I am writing to request approval for a substantial amendment to the following research study for which I am PI:

Study title: A novel field-based performance test to assess neuromotor control during diverse movement sequences of varying intensity in U21 high performance football-code athletes

Ethics Reference: AM151202-06

The originally approved study was to examine responses to an acute HIIT training in U21 footballers from Southampton Football Academy. An amendment to the ethics for this study was granted to use the exercise protocol in sedentary premenopausal women.

The reason for requesting a further amendment is twofold:

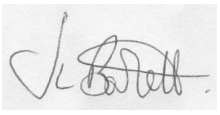
1. It is proposed to test additional youth male footballers to increase participant numbers and using a newer generation motion tracking device (Catapult), with a higher sampling frequency, used during two subsequent training studies which will form the third and fourth results chapters of the thesis. Testing a larger cohort of youth players with this system will create consistency across all motion tracking data gathered during the thesis.

2. Extend the end date to the previously approved amendment to test premenopausal women with the HIIT exercise protocol to enable further recruitment.

Changes to previously approved documents (PIS and PSQ) are highlighted in the documents attached in support of this amendment request.

Please do let me know if you need any further information before making a decision.

Yours sincerely



Dr JL Bowtell

Associate Professor

A2.3 Approval of Ethics: Acute Diverse HIIT

Sent: 30 June 2016 10:11 **To:** Bowtell, Joanna **Subject:** Amendment request: AM160712-31 - 140618/A/01 - Jo Bowtell

Dear Jo,

I am pleased to advise that your application for an amendment to the above study, involving the under 21 football study has been approved.

Please keep this email with the original approval certificate.

Best wishes,

Rosa

Sally Discombe

Ethics Committee Administrator

Sport and Health Sciences

College of Life and Environmental Sciences

University of Exeter

E: s.j.discombe@exeter.ac.uk

T: +44 (0)1392 722884

A2.4 Approval of Ethics: Acute And Chronic Diverse HIIT in Female And Male Dancers

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road
Exeter
EX1 2LU
United Kingdom

Certificate of Ethical Approval

Proposal Ref No: B 140618-01

Title: Movement-Specific High Intensity Interval Training (MS-HIIT) for dancers in professional training: An Intervention Study in students from London's Central School of Ballet

Applicants: Dr Joanna Bowtell (Associate Professor, Deputy Head of Discipline,SHS), Associate Professor Karen Knapp (Senior Lecturer, CEMPS), Dr Jon Fulford (Post Doctoral Research Fellow in MRI) Suzanne Scott (PhD student)

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until October 2016.

Signature: Mark Wilson Date: 21/07/14

Name/Title of Ethics Committee Reviewer: Dr Mark Wilson

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 3. Diverse-Movement (DM)-HIIT Protocols

A3.1 Acute responses to DM-HIIT (Chapter 6)

The link below is to a private you tube channel where the protocol used to examine acute effects of DM-HIIT in sedentary females and female dancers can be viewed.

https://www.youtube.com/playlist?list=PLNv5kvrpfCbJFt8k55DDWm2WHG4Wc0kQH

A3.2 Home exercise DM-HIIT (Chapter 7)

The following web address can be used to access a private you tube channel, through which familiarisation, Ramp 1 (weeks 1 – 6) and Ramp 2 (weeks 7 – 12) of the home exercise intervention with DM-HIIT (Chapter 7) can be viewed.

https://www.youtube.com/playlist?list=PLNv5kvrpfCbKzQAsu7Je9C5HAPRnBSVAx

A3.3 DM-HIIT in male and female dancers (Chapter 8)

Using the link below, it is possible to view the exercise protocol implemented at baseline and after 12 weeks testing in male and female dancers supplemented with DM-HIIT. The link also provides access to view ramp 1 (weeks 1 - 4), ramp 2 (weeks 5 -8) and ramp 3 (weeks 9 – 12).

https://www.youtube.com/playlist?list=PLNv5kvrpfCbJFt8k55DDWm2WHG4Wc0kQH

Appendix 4. Author's contribution to studies

The author's contribution to studies described in this document are summarised in Table 41. Research was conducted collaboratively, co-researchers on each study are listed below.

A4.1 Study 1

Effects of 16 weeks Small-Sided Football or Whole-Body Vibration on Bone - A Randomised Controlled Intervention with Brief Diverse Exercise in Sedentary Premenopausal Females (Chapter 4)

Research team

Professor Joanna Bowtell; Dr Luke Connolly; Dr Rosemary Davies; Georgious Ermidis; Dr Jon Fulford; Dr Susan Hopkins; Dr Sarah Jackman; Ross Julian; Associate Professor Karen Knapp; Professor Peter Krustrup, Rebecca Lear; Dr Jude Meakin, Professor Magni Mohr.

Biomarker analyses University of Copenhagen; Jamie Blackwell, School of Sport and Health Sciences, University of Exeter

A4.2 Study 2

Development of Brief Diverse Movement High-Intensity Interval Training (DM-HIIT): The Grid Feasibility Study (Chapter 5)

Research team

Professor Joanna Bowtell; Dr Jon Fulford; Associate Professor Karen Knapp; Jock Scott

Biomarker analyses Jamie Blackwell, University of Exeter

A4.3 Study 3

Characterising Locomotor Profile and Acute Physiological Demands of Diverse Movement-HIIT (DM-HIIT) In Sedentary Women And Female Dancers (Chapter 6)

Research team

Professor Joanna Bowtell; Anna Brodrick-Turgoose; Dr Luke Connolly; Stephanie D'Ath; Dr Jon Fulford; Associate Professor Karen Knapp; Jock Scott

A4.4 Study 4

DVD The Effects of 12 Weeks Diverse HIIT On Acceleration Profile And Bone Metabolism: A Randomised Controlled Home Exercise Intervention In Sedentary Premenopausal Females (Chapter 7)

Biomarker analyses Markers of bone turnover: Dr Jon Tang & Professor William Fraser, University of East Anglia; CRP: Jamie Blackwell, University of Exeter

Research team

Dr Stephen Bailey; Professor Joanna Bowtell; Dr Luke Connolly; Dr Jon Fulford; Professor Andrew Jones; Associate Professor Karen Knapp; Jock Scott

A4.5 Study 5

Effects of 12 Weeks Diverse HIIT On Acceleration Profile In Young Male and Female Ballet Dancers (Chapter 8)

Research team

Professor Joanna Bowtell; Anna Brodrick-Turgoose; Dr Luke Connolly; Stephanie D'Ath; Dr Jon Fulford; Associate Professor Karen Knapp; Jock Scott

Biomarker analyses Biomarkers of bone turnover: Dr Jon Tang & Professor William Fraser, University of East Anglia; gonadal steroids, serum 25(OH)D: Dr Tim McDonald, Royal Devon and Exeter NHS Hospital

Table 41 Summary of Author Contribution to Studies

Study	Phase 1 -study design, funding application, ethics procedures	Phase 2 -recruitment, assessment of eligibility and consent, group allocation	Phase 2 -baseline data acquisition & participant profiling	Phase 3 -conducting intervention, ongoing data acquisition, participant liaison, support & advice	Phase 4 -exit data acquisition, follow-up with participants	Phase 5 -data analysis	Bms	Phase 6 -writing up, publication, dissemination & presentation of results
Study 1 Chapter 4	--	✓	✓+⌂	✓	✓+⌂	✓	--	✓
Study 2 Chapter 5	✓	✓	✓	✓	✓	✓	--	✓
Study 3 Chapter 6	✓	✓	✓	✓	✓	✓Ŧ	--	✓
Study 4 Chapter 7	✓	✓	✓⌂	✓	✓⌂	✓Ŧ	--	✓
Study 5 Chapter 8	✓	✓	✓	✓	✓	✓Ŧ	--	✓

Note. ✓ Indicates author contribution; ✓+ not including dual x-ray absorptiometry (DXA); ✓⌂ not including MRI; ✓Ŧ not including data scripts for parsing accelerations according to threshold.
Bms: Biomarker analyses involving assays.

Appendix 5. Dissemination of research findings

A5.1 Bridging The Gaps 23 September 2013

University of Exeter and EPSRC jointly funded initiative

Oral presentation showcase (delivery: Associate Professor Karen Knapp)

'Dance Grid Feasibility Study: Movement Training For Health' Scott, S.

A5.2 International Society for Magnetic Resonance in Medicine (ISMRM) 8th – 9th November 2014

Poster presentation

'Metabolic Adaptations in Muscle after Short Bout Exposure to Recreational Football: An Intervention Study in Sedentary Pre-menopausal Women'

Scott, S., Connolly, L., Jackman, S., Fulford, J., Knapp, K., Bowtell, J., Davies, R., Meakin, J., & Krustrup, P.

A5.3 National Osteoporosis Society Conference (NOS) 30th November – 2nd December 2014

Poster presentation

'Movement Training for Bone Health: The Dance Grid Feasibility Study'

Scott, S., Fulford, J., & Knapp, K.

A5.4 National Osteoporosis Society Conference (NOS) 7th November – 9th November 2016

Poster presentation

'Serum Vitamin D Status in Elite Male and Female Dancers: A 12 week Observational Study'

Scott, S., Knapp, K.M., Brodrick, A., Fulford, J., Krustrup, P., & Bowtell J.L.

A5.5 International Association for Dance Medicine & Science (IADMS)

25th – 28th October 2018 28th International Conference

Oral Presentation (15 minutes)

'Calcaneal Heel Stiffness, Vitamin D and BMI – an Observational Study in Young Classical Dancers

Scott, S., Brodrick, A., De'Ath, S., Fulford, J., Krustrup, P. K., Knapp, K,M., & Bowtell J.L.

A5.6 European College of Sports Science (ECSS)

4th – 7th July 2018

Oral Presentation (15 minutes) Delivered by Professor Joanna Bowtell

'Vitamin D Profile, Sex Hormone Status and Biochemical Markers of Bone Turnover in Female Dancers: a 12 week HIIT Intervention Study'

Scott S., Knapp K.M., Brodrick A., Fulford J., Krustrup K., Tang J., Fraser W. & Bowtell J.L.

A5.7 Publications

Jackman, S. R., Scott, S., Randers, M. B., Ørntoft, C., Blackwell, J., Zar, A., Helge, E. W., Mohr, M., & Krustrup, P. (2013). Musculoskeletal health profile for elite female footballers versus untrained young women before and after 16 weeks of football training. Journal of sports sciences, 31(13), 1468-1474.

Bowtell, J. L., Jackman, S. R., Scott, S., Connolly, L. J., Mohr, M., Ermidis, G., Julian, R., Yousefian, F., Helge, E. W., Jørgensen, N. R., & Fulford, J. (2016). Short duration small sided football and to a lesser extent whole body vibration exercise induce acute changes in markers of bone turnover. BioMed research international, 2016.

Connolly, L. J., Scott, S., Mohr, M., Ermidis, G., Julian, R., Bangsbo, J., Jackman, S. J., Bowtell, J. L., Davies, R. C., . . Hopkins, S. J., &

Seymour, R. (2014). Effects of small-volume soccer and vibration training on body composition, aerobic fitness, and muscular PCr kinetics for inactive women aged 20–45. Journal of Sport and Health Science, 3(4), 284-292.

Connolly, L. J., Scott, S., Morencos, C. M., Fulford, J., Jones, A. M., Knapp, K., Krustrup, K., Bailey, S. J., & Bowtell, J. L. (2020). Impact of a novel home-based exercise intervention on health indicators in inactive premenopausal women: a 12-week randomised controlled trial. European journal of applied physiology, 120(4), 771-782.

A5.8 Training

IRMA. Birmingham, 2012

Venipuncture, cannulation, PGR introduction to STATA and SPSS statistics. University of Exeter, 2012 – 2015

'Teamsports - Methods and Research'. Post-Graduate courses 2012 & 2014, University of Copenhagen, DK

'MR in musculoskeletal imaging for sports-injuries'. ISMRM, Milan 2014.

A5.9 Funding awards

2012 – 2014 Funding for tuition Part-time PhD, University of Exeter, studentship grant from FIFA, received from Professor Peter Krustrup.

Bridge The Gaps, May 2013 . University of Exeter and EPSRC joint initiative: funding to conduct Dance Grid Feasibility Study in sedentary females.

Assay of biomarkers of bone turnover (Study 4 and 5), Profesor Peter Krustrup, awrded from the University of Odense, as part of research collaboration.

Glossary

Code (e.g. dance code, athlete code)	A term to denote a performance discipline, within competitive sport or aesthetic practice, which has internally consistent conditions, such as rules of play, or a shared grammar of activites and practice, such as in classical dance choreography and training
Cyclic (acyclic)	Describes activities, such as locomotor gait, characterised by recurring and stereotypical or similar phases of actions
Diverse movement	Denotes exercise actions which are not uni-directional or uniform in the loading they provide to the body
Leanness emphasis	Used to indicate a sport or aesthetic discipline in which a slender or lean physique may confer a competitive advantage, such as a jockey in professional horse racing, athletes in sports where a weight class is prescribed, such as competitive weight-lifting and boxing, or a dancer required to conform to a tradition of physical appearance or to convey a quality of lightness
Monotonic, non-monotonic	Term describing a quality of movement that is characteristically unvaried in speed in or emphasis. Quantatitvely it is described by actions such as those of gait which are recur in a similar temporal framework and during which speed is unchanging or only minimally altered and has a quantitative parallel in (i.e. absence or reduced presence of accelerations)
Somatotype Somatotypically lean	A reference to the external appearance of the body which includes qualitative descriptors, such as lean (denoting slender, in reference to dancers) rather than exclusively quantitative indices such as body weight or composition
Team sports	A form of organised group activity that is competitive, involving a few or multiple players organised as teams. Rules of play depend on the sport and activity is conducted on court or field surfaces, indoors or outdoors