RESEARCH PROTOCOL

Sheffield Hallam University

Local study number: Ethics Review ID: **ER52048815**

Protocol Version and Date: Version 5.0 30th June 2024

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Section 1: Summary of Protocol Details

Title of project:

The influence of pre-exercise feeding on acute bone turnover biomarkers in response to physical activity in healthy participants

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1.3 Protocol version number and date: 5.0, 30th June 2024

Section 2: Research question(s)

1. Does timing of feeding around a mechanical loading stimulus to the skeleton alter the response of bone turnover to exercise?

Section 3: Abstract

The benefits of increased human lifespan depend upon the duration of healthy, independent living; the health span. Bone-wasting disorders contribute significantly to loss of independence, frailty, and morbidity in older people. Therefore, there is an unmet need globally for lifestyle interventions to reduce the likelihood of bone fractures throughout the life-course and with ageing. Although many mechanisms are involved in disorders of bone loss, there is no single regulatory pathway and, therefore, there is no single treatment available to prevent their occurrence.

We have previously shown that in young 17-week-old mice, 16-hour fasting followed by reintroduction of food for 2 hours increased markedly the potency of mechanical loading, which mimics the effect of exercise, to induce new cortical bone formation (Samvelyan et al, 2021). Consistent with this finding, fasting, and re-feeding increased the response of bone to a loading stimulus that, alone, does not stimulate new bone formation in ad-lib fed mice. The hormones, including insulin, that change in response to timing of feeding have osteogenic effects that interact with loading-mediated effects. Our findings indicate associations between timing of food ingestion and bone adaptation to loading.

Our aim in these studies is to determine whether fasting/feeding interventions alter the effect of mechanical loading on bone anabolic activities and increase bone mass. Such non-pharmacological lifestyle interventions may benefit skeletal health of humans throughout life-course and in older age.

Section 4: Aims and objectives of the study

The overall aim of this study is to determine whether an adequate mechanical stimulus to the skeleton and the timing of feeding, could improve the balance of bone formation to bone resorption and alter bone turnover in young adults. The primary endpoint of this study is the change of the bone resorption biomarker C-terminal telopeptide of type-1 collagen (CTX-1) expression between pre-exercise and 2 hours following the exercise timepoints.

Objectives:

- To compare the effect of exercise on bone turnover markers before and after the meals following overnight fast in young adult male participants
- To assess the synergistic effects of feeding in addition to exercise on bone formation and resorption biomarkers in young adult male participants
- To determine the concurrent role of osteotropic hormone insulin in bone homeostasis in young adult male participants

Section 5: Background and rationale

5.1 Scientific background and relevance

The dramatic increase in human life expectancy in the last 60 years has been driven by reduced deaths of people in their 60s and 70s, leading to demographic shifts so that most populations globally are characterised by increasing numbers of elderly adults. However, an increasing number of these extra years of life are spent in poor health with more years spent in care facilities due to reduced ability to live independently. Osteoporosis is a major contributor to loss of independence due to bone fractures and resulting hospital treatments lead to significant morbidity. Even after successful fracture treatment, independent living is compromised in many patients. While drug treatments reduce consequences of osteoporosis significantly, there is a pressing need for non-pharmacological interventions to improve bone health across the life-course and to reduce likelihood of age-related bone disease.

The skeleton has protective, mechanical and metabolic roles, providing structural support and acting as a site for mineral storage (Seeman et al., 2006). Bone turnover, the cellular machinery responsible for bone integrity and strength, is a finely balanced process that is responsive to hormones and mechanical loading.

Physical activity is beneficial for the skeleton acting through the process of functional adaptation, a process that is less effective in older animals and humans than younger ones (Samvelyan et al., 2021). In addition, older people and those with bone loss, who are already at increased risk of fractures, may be

unable to perform exercise that is sufficiently vigorous to strengthen their bones. Therefore, there is a need to identify the most effective ways to exercise with maximal benefits to the musculoskeletal system.

Exercise is a non-pharmacological intervention that can modify bone turnover, improve bone health and reduce the risk of osteoporosis in later life (Bonnet and Ferrari, 2010). Mechanical loads that are produced by exercise change local microenvironments of the canalicular networks within the bone framework via dynamic fluid shifts stimulating local osteocytes and ultimately bone turnover (Schaffler et al., 2012; Klein-Nulend et al., 2013).

The complex and coordinated relationship between the endocrine regulation of energy metabolism, adipose tissue, and bone homeostasis has been studied extensively (De Paula et al., 2013). Concentrations of hormones, including insulin and amylin, change profoundly in anticipation of, during or after eating (preprandial, prandial, and postprandial responses). These hormones have potential anabolic effects on bone homeostasis, therefore, synergies between endocrine and mechanical effects could alter the bone turnover response to exercise.

The measurement of bone turnover markers, such as procollagen type 1 N-terminal propeptide (P1NP) and CTX, allow to assess the dynamic fluctuations in metabolic activity of bone (Delmas et al., 2006). Prolonged endurance exercise increases CTX (Guillemant et al., 2004; Hermann et al., 2007; Kerschan-Schindl et al., 2009; Maïmoun et al., 2006; Scott e al., 2010) but bone formation markers are largely unresponsive (Guillemant et al., 2004; Hermann et al., 2007; Maimoun et al., 2006; Scott e al., 2010). This suggests that prolonged exercise might result in a transient negative bone remodelling balance and this acute response may be implicated in changes in bone mineral density with repeated exercise over time in some sub-groups. However, the response of bone turnover markers to exercise is highly variable and appears to be modality-, intensity-, age- and sex-specific.

Feeding modulates the metabolic responses to prolonged exercise. The metabolic adaptations to fuel supply when fed and with fasting are well studied, however, there is limited consideration of the effect of these practices on other biological responses to exercise, such as bone metabolism. Fasting attenuates the circadian variation in CTX, a marker of bone resorption, whilst feeding suppresses resting CTX within 1-hour and concentrations are decreased by around 50% after 2–3 hours (Bjarnason et al., 2002; Henriksen et al., 2003; Holst et al., 2007). In contrast, and in line with the response to exercise, bone

formation is largely unresponsive to nutrient ingestion (Bjarnason et al., 2002; Henriksen et al., 2003; Holst et al., 2007). Concentrations of hormones including insulin and parathyroid hormone (PTH) change profoundly across pre-prandial, prandial, and postprandial states. These hormones have potential anabolic effects on bone homeostasis, therefore, synergies between endocrine and mechanical effects could alter the bone turnover response to exercise.

Despite the reported independent effects of feeding and exercise on bone metabolism, only a limited number of studies in humans have investigated how feeding and fasting practices mediate the effects of endurance exercise on bone metabolism, with mixed findings (Borer et al 2019; Scott et al., 2012). Existing studies in this area have largely been carried out on athletes (Scott et al., 2012) or populations suffering from different disease states, such as T1 and T2 diabetes (Borer et al., 2019). Previous studies have also not always used a comparator/control arms (Scott et al., 2012).

The flat running protocol used in previous studies (Scott et al., 2012) may also not be optimal to induce enough strain on bone for bone turnover markers to fully respond and therefore in designing the study, mechanical skeletal loading will be enhanced by using a downhill treadmill running protocol. The idea regarding loading is based on a study where running at 3 m/sec at a 6° decline increased ground reaction force (GRF) by 24.3%, while running on a +6° incline reduced it by 22% relative to exercise on level surface (Gottschall and Kram, 2005). This proof-of-concept study will investigate the effect of an overnight fast versus feeding of a single mixed meal, on the acute changes in bone turnover markers to a bout of treadmill running in healthy young adults.

5.2 Study rationale

Since dietary interventions are associated with healthy ageing (Kalache et al, 2019), a combination of exercise and nutrition may benefit bone health in ageing by utilising synergies between osteotropic influences. There is already support for this idea. The data from Samvelyan et al (2021) latest study on model organisms provides evidence that the effects of anabolic hormones in increasing bone formation in response to mechanical loading may be manipulated by changing timing of feeding in relation to exercise imposition (Samvelyan et al, 2021). There are already widespread fasting and caloric restriction interventions in use in humans for weight control and for healthy ageing. For instance, the 5:2 diet in which food intake is restricted to 500–600 calories on 2 days per week, which could be exploited to provide fasting experienced by animal models in Samvelyan et al (2021) study before eating and then exercising. It has also been shown that in diabetic postmenopausal women there is a significant increase

in bone osteogenic response (defined as the ratio of osteogenic C-terminal propeptide of type I collagen (CICP) over bone-resorptive CTX markers) when exercise followed a meal but not when it preceded a meal (Borer et al., 2019). Further, bone turnover markers and clinical risk factors can be used to identify patients at risk of osteoporotic fracture as well as those who have secondary osteoporosis (Clowes and Eastell, 2000).

5.3 Clinical relevance

Osteoporosis is a major contributor to loss of independence due to bone fractures and resulting hospital treatments lead to significant morbidity. While pharmacological treatments can reduce consequences of osteoporosis, there is a pressing need for non-pharmacological interventions to improve bone health across the life-course and to reduce likelihood of age-related bone disease.

This study will allow us to determine whether synergistic potentiating effects on bone metabolism are observed in humans in relation to the timing of food ingestion and what recommendations can be made to people with respect to food ingestion and exercise to obtain the maximal bone benefits from aerobic exercise. Furthermore, if we can identify interventions that potentiate bone's response to aerobic exercise in younger adults, then such interventions may have the ability to maximise bone mass in younger adults so that with ageing, the additional bone mineral density and improved bone microarchitecture would extend the time before osteoporotic “fracture thresholds” are reached. Therefore, understanding the bone metabolic response to exercise following fasting and feeding in younger adults is important.

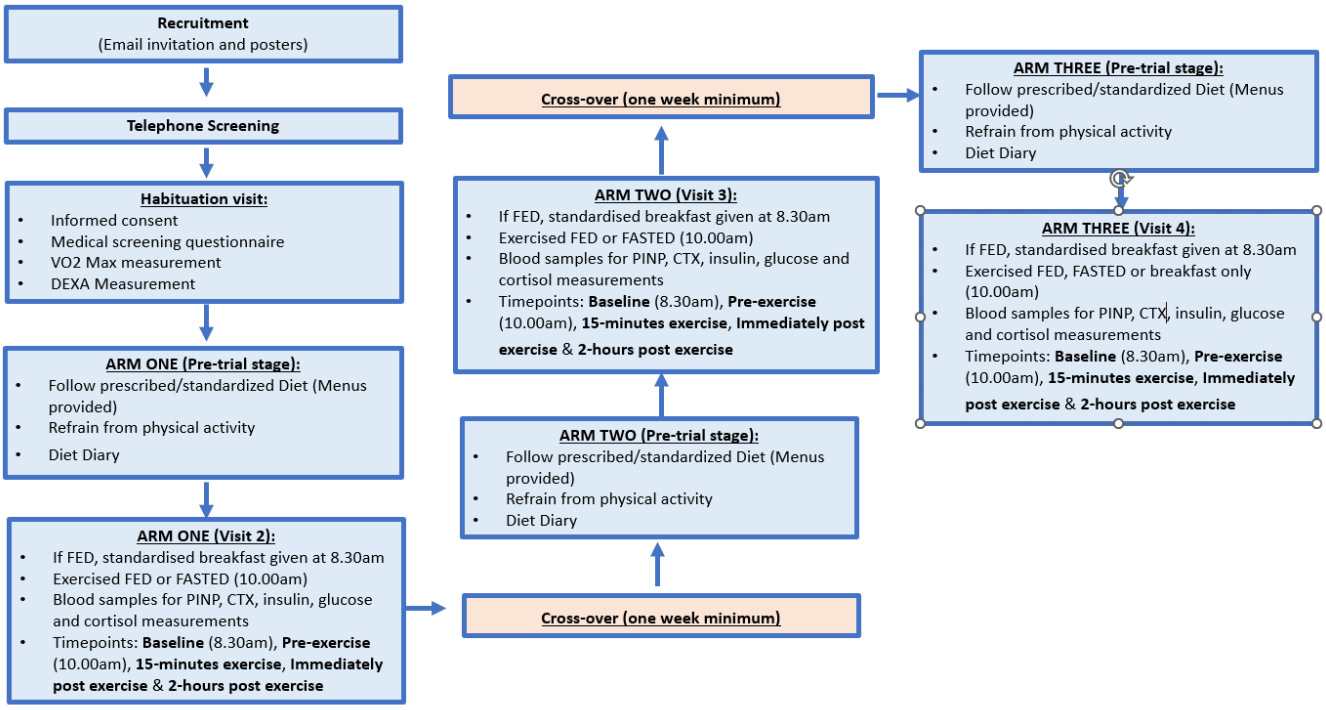
Section 6: Plan of investigation

6.1. Study design

In this proof-of-concept study, sixteen young adult men (age: 28 ± 4) will be recruited in good physical condition. This will be a three-arm randomised cross-over study with a 1-week washout period between visits (**Figure 1**). Participants will be exercised following a 12-hour overnight fast (FAST) and exercised 90 minutes after a meal (FED) to test the temporal sensitivity of the loaded bone to absorbed nutrients. Participants will also attend a breakfast-only arm (without the exercise protocol). The meal will consist of 60% carbohydrate, 25% fat and 15% protein. The exercise will be a downhill running protocol adapted from previous studies in this area (Scott, 2012; Borer, 2019). Counterbalancing measures will be put in place to prevent and minimise order effects.

Participants will undergo a baseline assessment for health history (PAR-Q). Baseline measurements of weight, height, BMI and maximal oxygen uptake as well as baseline bone mineral density and body composition scans of participants will be performed with a dual energy X-ray absorptiometry (DXA) to characterise the study sample.

Figure 1: Study Design



6.2 Power calculation

The primary outcome of this study is the difference in change in CTX concentration between the pre-exercise and 2-hour post-exercise timepoints between the fasted-exercised and fed-exercised arms. Based on changes in CTX from a previous study (Scott et al., 2012), we designated 30% as a minimally significant difference and that 30% would be approximately 0.168 µg/l. We calculated a standard deviation of 0.2 µg/l based on results from our previous studies with serial measurements of CTX. To achieve 80% power for demonstrating this mean difference as statistically significant at the 2.5% two-sided level requires a total of 16 participants (including 2 additional participants for withdrawals). This sample size is in line with that of other studies in this area.

6.3 Study measurements

The blood samples will be collected (10ml) by cannuala at baseline, immediately pre-exercise, and after 15 minutes and 45 minutes of exercise and after 2-hours of recovery. The osteogenic response to exercise will be determined by measuring a marker of bone formation; P1NP and a marker of resorption; CTX-1.

To assist in making inferences on the possible hormonal interactions with mechanical, cardiovascular, and psychological stress in our study, and thus their contribution to changes in bone markers, we will also measure plasma concentrations of cortisol; a marker of physiological and psychological stress and because of its promotion of bone resorption. Insulin and glucose measurements will allow us to assess the change of concentrations in fasted and fed states. Urine samples will be collected at baseline, immediately before and after the exercise. Samples will be frozen at -80^{o}C and analysed for bone turnover biomarkers later with the permission of participants.

6.4 Methods

6.4.1 VO_{2Max} and determination of running speed

Participants will perform a two-stage graded exercise test on a treadmill to determine the speed of downhill running that elicits 70% VO_{2max}. For the first stage, VO_{2max} will be determined via a graded exercise test. The test will begin with participants completing a 4-min warmup at 4.8km/h, followed by 1 minute of passive rest. The graded exercise test will start between 8.0 and 9.6 km/h, with the velocity increasing by 1.6km/h every 2 mins until reaching a speed of 14.4km/h. At this point, incline will be increased by 2% every 2 mins until the participant reaches volitional exhaustion (Succi et al. 2022). Heart rate and rate of perceived exertion (RPE) will be recorded via heart rate monitor (Garmin HRM-Dual, Garmin, KS, USA) and RPE Scale (Borg, 1982), to confirm maximal effort. Expired air will be collected and the gas exchange will be analysed using the Metelyzer 3B (Cortex, Leipzig, Germany). The equipment will be calibrated prior to each test as per manufacturer's directions using standard gases.

The second stage of the treadmill test will be used to determine the running speed that elicits 70% VO2peak at a decline of -6%. Participants will start running at the same speed as the first test. Speed will be increased by 1km every 2 mins for five stages. Regression analysis will then be performed to determine the speed that elicited 70% VO_{2max}. Participants will then rest for 30 minutes.

5.4.2 Familiarisation

Once running speed has been determined, the participants will then be familiarised with the downhill running protocol to be used in the experimental visits. This familiarisation visit will expose the participant to the eccentric strain and the subsequent muscle damage associated with downhill running (Southall-Edwards, et al. 2022). As muscle damage can have a detrimental effect on markers of bone formation (Huang, et al. 2022), the prior exposure of the running protocol to the participants should blunt the muscle damage response in subsequent bouts of downhill running due to the repeated bout effect (Khassetarash, et al. 2022).

6.4.3 Downhill running protocol

Participants will perform 45 min of treadmill running at 70% VO_{2max} at a -6% slope, one and half hours after a standardised breakfast (FED) or fasted (FAST). Downhill treadmill slope will increase Ground Reaction Forces (GRFs) relative to level walking (Gotschall and Kram, 2005).

6.4.4 Bone Mineral Density (BMD) measurement

Bone mineral density and whole body composition scans will be performed at the familiarisation visit with a dual-energy X-ray absorptiometry (DXA) scanner (Hologic). The lumbar spine (L1 through L4), femoral neck, trochanter, and total hip areas will be scanned for determination of areal BMD (g/cm2).

6.4.5 Bone turnover markers, cortisol, insulin and glucose measurements

Blood samples (around 10ml) will be collected into serum-separation tubes containing spray-coated silica and polymer gel for serum separation (BD Vacutainer venous serum separation tubes: Hemogard, Fisher Scientific, UK) for determination of bone turnover markers PINP and CTX-1. Measurements of bone turnover markers will be done at Bone Biochemistry Laboratory (The University of Sheffield, UK). Measurement of glucose, insulin, and cortisol will be done at the Chemical Chemistry laboratory (Sheffield Teaching Hospitals, UK).

6.4.6 Sample handling

Samples will be collected into Serum Separating Tubes (SST). Blood in SST tubes will be left to clot for 15-30 minutes at room temperature and centrifuged at 2000 g for 10 minutes. The serum will be aliquoted and stored at -80^{0}C until analysis.

6.5 Setting for the project

Oversight of the study will be at Sheffield Hallam University. The study will be conducted at the Advanced Wellbeing Research Centre (Sheffield Hallam University, UK). The study will conform to all standards of Good Clinical Practice and will be overseen by an experienced academic.

The Principal Investigator is Dr Jasmine Samvelyan (Senior Lecturer in Biomedical Science, Anglia Ruskin University School of Medicine) and co-investigators for the study are Dr Simon Bowles (Senior Lecturer in Nutrition, Sheffield Hallam University)**,** Professor Eugene McCloskey (The University of Sheffield) and Professor Richard Eastell (The University of Sheffield). Professor Craig Sale (Manchester Metropolitan University) will provide academic support for study concept and design. Data collection (after appropriate training) will be the responsibility of Dr Jasmine Samvelyan registered at Anglia Ruskin University.

6.6 Participants

Participants will be healthy male adults aged 28 $\pm$ 4 years.

6.7 Recruitment

Participants will be invited to take part in the study by invitation email and by poster. Posters will be distributed around Sheffield Hallam City Campus Buildings. Invitation emails will be sent out to Food and Nutrition students studying on undergraduate and postgraduate Food and Nutrition courses within the Department of Service Sector Management.

Prospective participants will be followed up by telephone or email for screening to make sure that they meet the eligibility criteria for the study. Eligible participants will be sent an appointment letter to confirm their study visit.

6.7.1 Informed consent

All participants will give informed consent before enrolment on to the study. Each prospective participant will receive a participant information sheet giving details of the study and will have at least 24 hours to consider their participation in the study before attending their first study visit to give consent to participation. All participants will be given the opportunity to ask questions about the study before giving informed consent. Informed consent will be collected of each prospective participant by the principal investigator/co-investigator or another appropriately trained member of staff after training and

observation as assigned by the delegation log. Participants will also have the opportunity to ask any questions that they have during any of their study visits.

6.7.2 Inclusion/Exclusion criteria

Inclusion criteria will be:

- Male (females excluded to remove the interplay between menstrual cycle and bone turnover)
- Caucasian ethnicity
- Aged 28 ± 4 years
- Are physically active (meet the UK guidelines for physical activity of at least 150 minutes moderate intensity activity and/or at least 75 minutes of vigorous intensity activity per week)
- Otherwise, healthy, able and willing to participate and provide written informed consent

Exclusion criteria will include:

- Current smokers
- Excessive alcohol consumption (max 15 alcohol units/week)
- Any musculoskeletal injury/disabilities
- Any conditions known to affect bone metabolism (e.g. uncontrolled hyper-/hypothyroidism, hyperparathyroidism, hypo-/hypercalcaemia) or malabsorption syndromes (e.g. Crohn's disease, coeliac disease or inflammatory bowel disease).
- Taking any medication known to affect bone metabolism (such as glucocorticoids or bisphosphonates)
- Positive Covid-19 test within the last 8 weeks
- Suffered a fracture in the previous 12 months
- Sedentary status (see physical activity inclusion criteria above)
- Have been told by medical professionals that they should not take part in moderate to high intensity exercise
- Should not be a professional athlete or take part in significant competitive recreational activity (takes no more than 4 structured exercise sessions per week on average)
- History of diagnosed eating disorders

6.8 Outcome measures and participant involvement at each visit

All participants will be required to attend the Advanced Wellbeing Research Centre (AWRC) on four separate occasions.

At the **familiarisation visit** each participant will give informed consent. Consenting participants will complete a PAR-Q, undergo a VO_{2max} test, and DXA scan to measure baseline bone mineral density and body composition. Baseline anthropometric measures, including height, weight, and BMI will be taken. Participants will be asked to follow a standardised diet three days prior to their second study visit. Participants will also be asked to refrain from strenuous physical activity during this period and will be asked to record their food and drink consumption in a diet diary.

At the **second visit**, each participant will arrive after a 12-hour overnight fast.
Counterbalancing measures will be used to assign participants to either the FASTED-exercised, FED-exercised or breakfast only conditions. During the FED-exercised or breakfast only arms, a standardised breakfast will be given at 8.30am. Exercise in the FED condition will take place 1.5 hours after breakfast has been administered (10.00am). When in the fasted condition, participants will also begin exercise at 10.00am.

Blood samples will be taken by cannulation for bone turnover marker measurement and hormone measurement at baseline (8.30am), pre-exercise (immediately before exercise), 15 minutes during exercise, 45 minutes (immediately post-exercise) and 2 hours post-exercise. Timings will also be consistent in the breakfast-only arm.

At the **third and fourth visits**, following crossover periods, participants will be assigned to the alternative conditions and study measurements repeated as per second visit.

6.8.1 Summary of participant involvement by study time point

Study Time Point	Familiarisation Visit	First Pre-trial stage (3 days before visit 2)	Visit 2	Second Pre-trial stage (3 days before visit 3)	Visit 3	Third Pre-trial stage (3 days before visit 3)	Visit 4
Activity							
Informed Consent	X						
Demographic data	X						
Anthropometry (Height, weight & BMI)	X						
PAR-Q	X						
DXA Scan (Hip, Spine and Body composition)	X						
VO2Max Measurement	X						
Downhill Running Familiarisation	X						
Diet Diary		X		X		X	
Refrain from vigorous physical activity		X		X		X	
Follow standardised dietary advice		X		X		X	
Breakfast only (no exercise) **or** FED-exercised **or** FASTED-exercised condition			X		X		X
Blood samples for biochemical measurements			X		X		X
Urine Samples			x		x		x
Participant expenses given (£100 gift voucher)							X

6.8.2 Summary of biochemical measurements by study visit time point (for both fasted and fed conditions)

Study Visit Time Point	**Baseline** (8.30am)	**Immediately Pre-exercise** (10.30am)	**During Exercise** (15-minutes)	**Immediately Post-Exercise Completion** (45-minutes)	**2-hours Post-exercise**
Biochemical measurement					
CTX	X	X	X	X	X
PINP	X	X	X	X	X
Glucose	X	X	X	X	X
Insulin	X	X	X	X	X
Cortisol	X	X	X	X	X

6.9 Statistical analysis

Normality will be assumed if Shapiro-Wilk test is not statistically significant (P> 0.05). Where data is normally distributed, the data will be presented as mean and standard deviation (SD). Where data were not normally distributed, data will be presented as median and IQR.

Biochemical data will be analysed using a linear, mixed model (LMM), with factors 'condition' and 'time point' as fixed effects and 'participants' as a random, within-group effect. The assumptions of the LMM (homogeneity of variance and normality) will be investigated by examining the distribution of residuals and the pattern of residuals versus fitted values. Where non-normality or non- constant variance are observed, a log transformation will be applied to the data so that the assumptions are satisfied.

Where there is a significant main effect of *Time* but no significant *Condition* x *Time* interaction, each subsequent time point will be compared against 'Baseline' using a pooled mean from the three groups using Dunnett's test. If the *Condition* x *Time* interaction is significant, within each group, each time point will be compared against baseline using a Dunnett's test and groups will be compared to each other at all time points using the Student Newman-Keuls (SNK) test.

6.9.1 Data collection

Electronic source data forms will be used to collate all the collected data for each subject. Members of the research team will complete data entry into an electronic case report form from participant questioning and measurements. All data from the electronic case report forms and the measurement reports will be entered onto the study database (created in Microsoft Access) and the data will be stored according to the regulations of the Data Protection Act 2018. Data entry will be checked by a second operator at a different time point. Participant consent forms will be signed as a paper copy, scanned, and stored electronically. The paper copy will be destroyed. The electronic case report forms, other study documents (e.g. study log) and study database and will be protected by the standard University windows and network login passwords as well as a database login unique to the study. A full data-management plan will be submitted for the Converis ethics review. An electronic participant enrolment log also will be maintained to cross-reference subjects against study code. All samples and data will be linked anonymously before analysis. No publications will contain information that will be able to identify individual participants.

6.10 Project plan

We aim to carry out data collection between October 2024 and March 2025. Biochemical analysis on the samples will be performed by the end of 2024.

We will consider the study complete when all participants have completed both study data collection time points, all laboratory analyses have been performed, the data entered into a database and checked (i.e. database lock), statistical analysis is done and the final report is written.

Section 7: Project management

7.1 Study team

Principal Investigator: Dr Jasmine Samvelyan

Co-Investigators: Dr Simon Bowles, Professor Eugene McCloskey, Professor Richard Eastell, and Professor Craig Sale

Nutritional supervision: Dr Simon Bowles

Statistical support: All investigators

Local laboratory supervision: Brent Robbins (AWRC)

All investigators will be responsible for study design and study oversight. Dr Jasmine Samvelyan and Dr Simon Bowles will be responsible for the day-to-day running of the study including consent and overseeing study visits. Dr Jasmine Samvelyan will be responsible for the running of study visits and data collection. The electronic Investigator Site File (ISF) will contain a study delegation log which will list all staff involved in the study.

The PI and co-investigators will meet on a regular basis to review the progress of the study and to ensure that the project delivers its intended outcomes on time.

7.2 Amendments to the protocol

If it is necessary for the protocol to be amended, the amendment and/or a new version of the study protocol will be notified through the Converis system for local approval. If necessary, revised Participant Information Sheets will be prepared and approved through Converis before participants are provided with this new information and asked to re-consent.

Section 8: Expertise of the researcher and associated team

Jasmine Samvelyan, The School of Medicine, Faculty of Health, Education, Medicine and Science (FHEMS), Anglia Ruskin University is a Senior Lecturer in Biomedical Science, and Lead of the Musculoskeletal and Developmental Biology research group at Medical Technology Research Centre (MTRC) at ARU. She has 10-year extensive research experience in utilising cellular, molecular, and *in vivo* approaches to study musculoskeletal ageing. Dr Samvelyan has used mechanical loaded murine models to explore associations between bone homeostasis and energy metabolism and examine microarchitecture of bone in young adult and osteoporotic aged mice and has conceived the original idea of this study. This provides compelling evidence that she has the necessary skills, accrued knowledge and motivation to successfully carry out the proposed project.

Simon Bowles is a Senior Lecturer in Nutrition and a Registered Nutritionist. He has worked within the Food Subject Group at Sheffield Hallam University since 2014 as an associate Lecturer and then from 2018 as a full-time lecturer. He has worked in clinical research for almost 10 years. He has previously worked on a Department of Health policy research programme funded project investigating the effect of obesity and age on vitamin D metabolism. He was also Chief Investigator for a double-blinded randomised controlled trial, administering large bolus doses of vitamin D to vitamin D deficient postmenopausal women in order determine the safety of large bolus doses in older people.

Eugene McCloskey MD, FRCPI is Professor in Adult bone disease, an acknowledged expert in the fields of vertebral fracture definition, osteoporosis epidemiology as well as non-invasive assessments of bone strength and fracture risk. Professor McCloskey has published over 450 peer-reviewed articles, book chapters, guidelines, and reviews.

Richard Eastell MD, FRCP, FRCPath, FMedSci is a medical doctor and Professor of Bone Metabolism at the University of Sheffield. He is currently Director of the Mellanby Centre based at the University of Sheffield. Some of his recent contributions have been authorship on key papers describing new treatments for osteoporosis, such as tibolone, zoledronic acid, denosumab and lasofoxifene as well as addressing issues about safety of medications and provide guidelines to diagnose primary hyperparathyroidism, a common disorder resulting in high levels of blood calcium. Richard has published over 550 research papers, his work as a clinical investigator was recognised in 2014, by the Frederick C Bartter Award from the American Society for Bone and Mineral Research.

Craig Sale, Professor at Manchester Metropolitan University has spent over 20 years investigating the impact of exercise and nutrition on health and performance in humans, with a particular focus on the triggers for adaptations in bone and muscle. He has worked and published in the areas of bone metabolism and health, muscle metabolism and performance, genetics, sports nutrition, broader human nutrition, biomechanics, cognitive function, and environmental physiology. He is also committed to delivering real world impact of significance and reach through research, having experience of conducting research on a range of human participants, including elite level and recreational level athletes, military personnel, and members of the general population.

Khawla Ekrayem, is a Medical Doctor from Libya Medical School. She has obtained MSc in Public Health From The University of Sheffield and currently is Doctoral candidate at Sheffield Hallam University at Biomolecular Sciences Research Centre.

Section 9: Ethical issues

The protocol will only be implemented after favourable opinion has been received after ethics review via the Converis ethics review system at Sheffield Hallam University. The protocol will also be signed off by an approved Radiation Protection Expert appointed by the AWRC.

All study participants will receive an information sheet prior to attending the study visit and will give written informed consent at the study visit prior to undergoing the study procedures. Informed consent will be obtained in accordance with GCP procedures to ensure confidentiality, and privacy.

The security of the research data will be assured by acting in accordance with the 2018 Data Protection Act (General Data Protection regulation). A full data management plan will be submitted with the Converis review application. The study will be carried out in compliance with the protocol and GCP procedures as described in ICH GCP 1996 and the Declaration of Helsinki concerning medical research in humans (2008) are in place to ensure appropriate consent, confidentiality, and privacy.

Risk to the participants is minimal. Blood samples will be taken by trained phlebotomists. Consent to use and store the samples will be obtained according to the Human Tissue Act 2004. There are small risks from having cannula inserted and blood samples taken. For most people, cannula insertions for blood draws do not cause serious problems; however, they may cause a bruise or a small amount of bleeding or pain at the puncture site. Some people may feel faint. In very rare occasions infection may occur. The total volume of blood sampling will be small.

This study requires a single DXA scan at the familiarisation visit with a DXA scanner (Hologic). The exposure required by the study is additional to routine clinical care. The total protocol dose is 0.02 mSv. This is equivalent to 4 days of average natural background radiation in the UK Ionising radiation can cause cancer which manifests itself after many years or decades. The risk of developing cancer as a consequence of taking part in this study is <0.001 %, which is very low (See letter from Medical Physics Expert). For comparison, the natural lifetime cancer incidence in the general population is about 50%. All users of the DXA scanner will be fully trained and Ionising Radiation (Medical Exposure) Regulations (IR(ME)R) compliant. The protocol has also been approved by the Clinical Radiation Expert at the AWRC (see CRE approval letter).

There is also a small risk of injury to participants during the exercise procedure. There is also a small risk of participants feeling faint during the procedure. A first aider will be on site at the AWRC if needed in such instances. However, participants will be generally healthy and will need to complete a medical screening questionnaire before taking part in the study. Only healthy participants who meet the current UK physical activity guidelines will be recruited (participants who have been told by medical professionals

that they should not take part in moderate to high intensity exercise will be excluded). Appropriate risk assessments will be carried out with risk mitigating controls put in place.

Participant samples will be stored at the AWRC before being transferred to the -80^{0}C freezer on the 12th Floor of the Owen Building (Food and Nutrition Subject Group) at the end of data collection. Samples will be stored here until the completion of the study. Other data will be stored securely in accordance with the Data Protection Act 2018 and potentially used for future research (with the consent of participants).

Participants will be informed of their right to withdraw from study at any point.

Section 10: Methods for disseminating research results

It is intended that the results of this study will be published in a peer-reviewed journal as well as presented at national and/or international conferences. The data will be used as preliminary data for external grant application.

Section 11: Strategy for taking the work forward

This study is a pilot study and data collected will be used to form part of a wider programme of the study of the synergistic effects of feeding interventions on the response of skeleton to physical activity in young and elderly humans including females and the concurrent role of candidate osteotropic hormones in bone homeostasis.

Section 12: Costing the project

The project will be funded through Anglia Ruskin University Higher Education Quality-related Research (QR) funding, local SHU fieldwork funding and PhD funding, according to the breakdown below:

Expense	Amount per unit (£)	Total Cost (£)	Comments
Office consumables		£350.00	QR funding
Clinical consumables		£2,500.00	QR funding
Participant breakfast		£300.00	QR Funding
Technical training (e.g. Canulation training)		£700.00	SHU Fieldwork funding
Dr Jasmine Samvelyan		£0.00	Work planned time

Dr Simon Bowles		£0.00	Work planned time + SRfR time
Participant Inconvenience Payments	£100	£1,600.00 (16 x £100.00)	SHU Fieldwork Funding
Biomarker analysis (80 measurements x 3)		£5,000.00	Covered by QR funding
Hormone analysis (insulin and cortisol - (100 measurements x 2 each cross-over study))		£6,850.00	Covered by QR funding
Total		**£17,300.00**	

Section 13: Funding

The project is funded through ARU Higher Education Quality-related Research (QR) funding, and by internal SHU Fieldwork Funding.

Section 14: References

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Section 15: Other Information

Location of data collection:

Advanced Wellbeing Research Centre
Sheffield Hallam University
Olympic Legacy Park
2 Old Hall Rd
S9 3TU

Laboratory Details:

Sheffield Laboratory Medicine
Sheffield Teaching Hospitals NHS Foundation Trust
Northern General Hospital
Herries Drive
Sheffield
S5 7AU

The University of Sheffield
School of Medicine, Dentistry and Health
Department of Oncology and Metabolism
Firth Court
Western Bank
Sheffield
S10 2TN

Appendix A: Project Plan

		Year 1												Year 2	
Project Month	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Date	**Apr 24**	**May 24**	**Jun 24**	**Jul 24**	**Aug 24**	**Sep 24**	**Oct 24**	**Nov 24**	**Dec 24**	**Jan 25**	**Feb 25**	**Mar 25**	**Apr 25**	**May 25**	**Jun 25**
Month relative to recruitment start	-4	-3	-2	-1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10
Activity															
Study methodology															
Recruitment strategy															
Study Specific SOPs															
Study Database															
Project management meetings															
Documentation for Research Ethics application															
Submission to REC															
Staff Initiation															
Ethical approval expected															
Expected recruitment start date															
Recruitment															
Study visits															
Biochemical measurements															
Data entry															
Data cleaning															
Data lock															
Data analysis															
Reports/Abstracts/ Manuscripts															