CENTRE FOR SPORT, EXERCISE & OSTEOARTHRITIS RESEARCH VERSUS ARTHRITIS

Department for Health

UNIVERSITY OF BATH

Protocol: Effect of short-term negative energy balance with or without exercise on systemic biomarkers in patients with knee osteoarthritis

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INVESTIGATORS

Professor James Bilzon

Dr Enhad Chowdhury

Ms Rachel Deere

Mr Matthew Farrow

Professor Dylan Thompson

Dr Fiona Watt

Dr Ben Davies

ADDRESS

Department for health

University of Bath

Bath

BA27AY

Correspondence: J.Bilzon@bath.ac.uk

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Summary

Osteoarthritis (OA) is the most prevalent form of arthritis and one of the leading causes of chronic disability worldwide [1]. OA is now considered to be a low-grade inflammatory disease [2] characterised by the progressive degradation and loss of articular cartilage. Diagnosis and progression of OA has traditionally been the product of radiography, however more recently, biological markers (biomarkers) are being increasingly explored. Biomarkers are direct indicators of biological, pathogenic and pharmacological responses to interventions and represent acute changes in tissue adaptations long before such changes are evident in radiographic images of biological structures, particularly the cartilage [3]. Early identification of disease therefore offers a window of opportunity for therapeutic intervention.

Long-term weight loss studies consisting of diet and exercise manipulation have highlighted beneficial effects on inflammation and clinical outcomes in overweight persons both with [4] and without OA [5], and 3-4 weeks of exercise exposure has been shown to positively influence pain and function in those with symptomatic knee OA[6]. We hypothesise that by creating a short-term negative energy balance through a combination of caloric restriction and exercise [7] that we will be able to reduce inflammation in individuals with knee OA. We also aim to determine if a dietary energy restriction condition can also reduce inflammation. We will examine changes in systemic biomarkers associated with inflammation in those with early-stage knee OA in response to the short-term interventions. We will also assess the impact of the intervention upon self-reported measures of pain/function and performance in functional tests. Importantly, we will be able to determine any of these effects without the confounding factor of substantial weight loss present in longer-term interventions.

As well as tracking symptomatic pain, it is also relevant that we incorporate a range of experiential pain measures. Such measures are used to assess pain sensitisation which is a phenomenon reported in individuals who suffer with knee OA [8]. Pain sensitisation in the knee OA population is suggested to be caused by peripheral inflammation at the joint and systemic level altering the way that the central nervous system responds to tactile and noxious stimuli [9].

The study will include both male and female participants that fit the eligibility criteria. Participants' activity levels and dietary intake will be monitored for 7 days and each will then be randomised into 1 of 2 intervention groups. Both groups will complete a 4-week observational wash-in period for us to determine the stability of the biomarkers, with participants acting as their own controls. Both intervention groups will then reduce their caloric intake by 5000kcal/week over 4 weeks, and the second intervention group will have the addition of 5 moderate-vigorous intensity exercise sessions (based upon target ratings of perceived exertion) per week for 30 minutes using a cycle ergometer to determine the added effects of exercise.

Systemic biomarkers in Osteoarthritis

Despite evidence of improved patient related/clinical outcomes (i.e. pain and function) in knee OA research, changes in radiographic images fail to show in a large percentage of patients that take part in clinical trials, even those lasting up to 3 years [10]. This emphasises the need to develop biomarkers that are both sensitive to change and predictive of disease activity to enhance such trials, improve the area of OA phenotyping and to allow for an overall improvement in patient care with regard to disease management. Additionally, systemic rather than local (within the joint i.e., articular cartilage, synovial fluid) biomarkers are less invasive and easier to acquire.

Cartilage degeneration is a hallmark of OA and is attributed to several catabolic mediators including ADAMTS5, MMP-13, hypoxia-inducible factor 2α and complement component 5^{+}. Of these, MMP-13 (Matrix Metallopeptidase-13) has attracted the most interest and focus due to its significant over-expression in OA joints, with almost no detection in normal joint tissues [11]. MMP-13 acts primarily on type II collagen [12] but also on aggrecan [13].

The most influential group of biomarkers associated with inflammation in knee OA are well known but not limited to; IL-1β, TNF-α, IL-6, IL-17, and IL-18. These biomarkers act catabolically on the homeostasis of tissues forming the joints and are associated with the expression of matrix metalloproteinase (MMPs) (primarily MMP-1; 3 and 13)[14]. As previously detailed, MMP-13 has been the main enzyme from the MMPs family to be attributed to cartilage degeneration by acting on both type II collagen and aggrecan. MMP-1 and MMP-3

are also potentially relevant with MMP-1 suggested to participate in osteochondral changes seen in OA as determined by the expression of MMP-1 from subchondral bone cyst cells [15]. MMP-3 has been positively related to the severity of knee OA, with its expression being greater with increased severity [16] but its specific role in knee OA progression is unclear.

Biomarkers that are associated with MMP-13 expression, act either directly, or indirectly and either by inhibiting or increasing its expression. Two of the most influential cytokines associated with the increased expression of MMP-13 in in-vitro OA cartilage are IL-1β and TNF-α [17, 18]. On the other hand, IL-10, IL-4 and IL-13 are associated with cartilage repair and suppress/inhibit the expression of MMP-13 through IL-1β and TNF-α [19, 20] during in-vitro studies. However, there lacks evidence to support these findings in-vivo. Many of those biomarkers associated with MMP-13 expression have also been associated with knee pain and function. IL-1β [21] and TNF-α [22] have been positively associated with high WOMAC scores for each sub scale of pain, stiffness and physical function. Conversely, an infusion of IL-4-10 protein improves deficient joint loading in animals[23], used as a surrogate marker of pain. Although there is no direct evidence highlighting the relationship between IL-13 and clinical outcomes, it is associated with supressing IL-1β and TNF-α which as stated above, are both positively associated with increased knee pain and reduced function.

As well as those inflammatory biomarkers associated with MMP-13 expression, it is also essential to highlight and focus attention on the influence of two key biomarkers associated with the pathogenesis of knee OA: CRP and IL-6. CRP is a marker of low grade systemic inflammation and is well known to be positively associated with cartilage volume, disease progression [24] and local joint inflammation[25]. IL-6 is a primary regulator of CRP but is also directly associated with knee cartilage loss [26] and is a significant predictor of knee OA [27], but it is unclear of the role of either in the pathogenesis of knee OA.

Focusing on IL-6, CRP, IL-1β, and TNF-α, IL-4, IL-10 and IL-13 allows us to target biomarkers linked with both clinical outcome measures (i.e., pain and function) and early-stage disease progression (i.e. inflammation and cartilage degeneration).

Impact of diet and exercise on systemic biomarkers associated with Osteoarthritis.

Diet and exercise treatments are widely recommended through clinical guidelines with the aim of improving pain and functional ability [28], and it is well documented that the manipulation of diet and/or exercise, have beneficial effects on systemic inflammation [29, 30], especially in those who are overweight/obese. Despite this, the current scope of research around the effects of diet only and combined diet and exercise treatments on OA biomarkers lacks in both the number of available studies, and the biomarkers assessed in those studies [31]. Additionally, those studies that have induced a negative energy balance through diet and exercise manipulation have been long-term investigations whereas our study is focused on relatively short-term changes. This has the potential benefit of improving systemic inflammation while being relatively independent of the effect of substantial weight change (i.e. loss of >5kg often seen in longer term studies), which is known to be a critical factor that reduces systemic inflammation[32] and can also independently improve pain and functional outcomes.

Purpose of study

The primary aim of the study is to assess changes in the primary clinical biomarker of systemic inflammation (CRP) in individuals with knee osteoarthritis.

We hypothesise that a 4-week lifestyle intervention employing a negative energy balance will decrease systemic CRP concentrations.

Secondary outcome variables –

- Changes in systemic pro- and anti-inflammatory cytokines and chemokines associated with knee OA.
- Changes in fasted markers of metabolic function (glucose, NEFA, triglycerides, total/HDL cholesterol)
- Changes in self-reported functional and pain outcomes
- Changes in functional tests
- Changes in experimental pain measurements

Participants

Eligibility for participation in this research trial will be based on information and measurements gathered at a preliminary screening visit.

We will recruit participants based on the following eligibility criteria:

- Diagnosed with uni -or bi-lateral knee osteoarthritis. Clinically deemed to be appropriate for conservative treatment and not surgical intervention through Virgin Care.
- Male or females with clinically diagnosed and radiographically confirmed knee osteoarthritis. Exclusion of other possible causes of knee pain/arthritis determined based upon clinical reasoning.
- Aged between 45 to 69 years.
- Body mass index of ≥27.5 kg/m^2 (to ensure we are working with chronic, unresolved low grade inflammation not just the result of injury – those with a BMI of >25kg/m^2 have clinically raised levels of CRP compared with those with a BMI of <25kg/m^2 [33]).
- Average daily physical activity level (PAL - total energy expenditure/resting energy expenditure) of <2.00, to ensure the individual is not highly active.
- Have no physical impairment unrelated to the knee that would prevent safe participation and their ability to comfortably complete cycling exercise under sub-maximal conditions.

Inclusion and exclusion criteria

Inclusion	**Exclusion**
Male or post-menopausal females	Inability to undertake cycling exercise safely**
Clinically diagnosed with knee Osteoarthritis including radiographic confirmation	Use of prescribed anti-inflammatory medication***
Oxford Knee Score ≥20 to ≤35 for index knee	Current smoker [34] (or having quit <6months ago)
Aged 45-69 years	Has a diagnosis of diabetes or other metabolic disorder
BMI ≥27.5kg/m^2 to ≤40.0 kg/m^2	Use of other medications that might interfere with study outcomes
Daily physical activity level <2.00*	Recent (within last 3 months) change in body mass of >5%

	Currently engaged in an ongoing programme of physiotherapy treatment
	Currently on waiting list or having had surgery for Osteoarthritis on the index knee (prior OA related surgery permitted on other knee if this is greater than 12 months prior to enrolment)
	Having had major hip/knee surgery within preceding 12 months
	Individuals with a blood pressure of greater than 180/110
	Currently pregnant or intending to become pregnant during the study

*Assessed during a preliminary period of ≥7 days via accelerometer.

**Assessed during pre-screening via physical activity readiness questionnaire (PAR-Q+).

***Patients that occasionally use NSAIDS will be asked to use paracetamol as a 'rescue medication' if they experience pain during the trial, with a minimum of a 3-day washout period prior to the trial.

Recruitment

Patients will be recruited through Virgin Care acting as a patient identification centre:

1) Potentially eligible patients attending a Health Optimisation clinic for Osteoarthritis (for individuals with a BMI >30 kg/m^2 or current smokers) as part of services provided by Virgin Care will be provided with study information. These individuals have been previously clinically diagnosed with knee osteoarthritis by a physiotherapist or general practitioner before being referred to the Health Optimisation Clinic. Physiotherapists or exercise instructors delivering these classes will provide study information (Participant Information Sheet) to potentially relevant individuals based upon age, body mass and Oxford Knee Score as described in inclusion criteria above.

As part of this identification process, only the direct care team will interact with the patients to the point at which individuals are provided with study information.

Sample size

A previous intervention conducted in sedentary overweight and obese women using the same energy restriction model for 3 weeks[7], established an effect size of 1.22 for the change in CRP with dietary energy restriction and vigorous intensity exercise 5 x per week. Using this effect size, an α of 0.05, and a power of 80%, it is estimated that a sample size of 8 would be

required for a two-tailed paired comparison. However, as we would hypothesise that the effect of the diet only intervention would be less, due to the lesser energy restriction and independent impact of exercise training upon CRP, an estimated effect size of 0.85 is proposed. Using an α of 0.05, and a power of 80%, it is estimated that a sample size of 13 would be required for a two-tailed paired t-test. Assuming a dropout rate of ~25%, we would aim to recruit ~16 individuals in each intervention arm. Between-group comparisons will be exploratory in nature, to determine sample sizes of future studies comparing the efficacy of the conditions.

Group allocation/Randomisation

Following screening/baseline measures of those included, participants will be allocated to either intervention group 1 (Diet restriction) or intervention group 2 (Diet restriction + Exercise) by an individual outside of the research team who will have no interaction with participants. To protect against chance imbalance between the two groups, this will be conducted via minimisation accounting for participant sex, age, BMI, and Oxford Knee Score.

Outcome measures

Primary outcome variable

- Change in the primary clinical biomarker of systemic inflammation (CRP).

Secondary outcome variables

- Changes in in systemic pro- and anti-inflammatory cytokines and chemokines associated with knee OA.
- Markers of metabolic function (glucose, NEFA, triglycerides, total/HDL cholesterol)
- Self-report measures of physical function and knee related symptoms
- Physical Activity Level
- Functional tests
- Pain measurements

Study overview

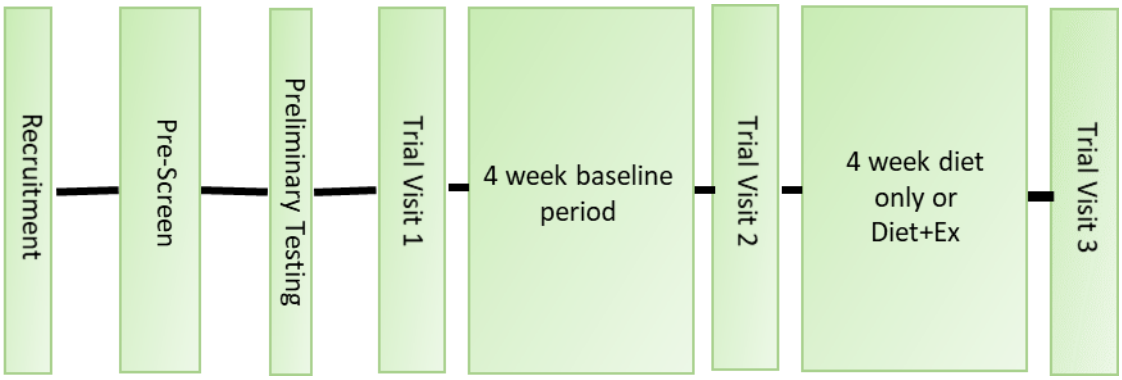

Figure 1. Study Schematic - General overview (detailed information on page 13)

Study design

Pre-screen

After potential participants have been recruited, they will be invited to the University of Bath for eligibility assessment and pre-screening following the provision of written informed consent. We will determine if individuals meet all inclusion criteria or any of the exclusion criteria through a screening questionnaire and measurement of BMI and resting blood pressure. We will also ask individuals to undertake the Oxford Knee Score questionnaire to allow us to use this data as part of our participant allocation. Participants will each be fitted with a physical activity monitor (Actiheart^{TM}) attached to the chest with a strap or 2 adhesive ECG pads. Participants will wear this physical activity monitor for 7 days. This data will allow us to calculate participant physical activity level (PAL) to ensure they meet the inclusion criteria for this measurement (i.e., <2.00).

Preliminary testing

The purpose of preliminary testing is to allow us to characterise the sample population based on anthropometric and fitness characteristics. We will note participant's body mass (kg), height (cm) and BMI (kg/m^2) and ask them to complete a submaximal exercise test on a cycle ergometer with expired gas analysis to predict maximal aerobic capacity. This will allow us to predict HR_{max} and VO_{2max} so that we can estimate the intensity at which individuals have been working during their training periods.

Pre-trial visits

In the 24 hours before trial days, participants will be required to avoid exercise and alcohol consumption. They will also be asked to record their food and drink intake in the 24 hours before the first trial and repeat this prior to subsequent visits. Participants will be asked to arrive at the laboratory fasted for ≥10hrs in the morning. Along with university policy, we will also call participants 24hrs before their visit to complete the COVID-19 Research Participant Screening Form.

Trial visits

All participants (regardless of intervention group) will attend 3 separate trial visits in which the following measurements/tests will be performed.

- DEXA scan + anthropometrics
- Resting metabolic rate (RMR)
- Venous blood sample (for collection of plasma and serum)
- Functional tests
- Self-report measures
- Experimental pain measurements

Wash-in period

Following trial visit 1, participants' will complete a four-week wash-in period (allowing participants to act as their own control). Throughout the wash in period, participants will be asked not to make any substantial changes to their normal lifestyle. Food diaries and activity monitoring will also be completed for 1-week of the wash-in period to enable prescription of the energy restriction diet. At the end of this 4-week period, participants will return for another trial visit to determine the stability of biomarkers and other outcomes under stable lifestyle conditions.

Intervention group 1

For the 4-week intervention period, participants in intervention group 1, will reduce their habitual diet by 5000 kcal per week, prescribed individually based on their 7-day food diary

and physical activity monitoring (i.e., energy intake and energy expenditure). The diet restriction will be based on an absolute energy deficit achieved by the intake of smaller food portions. This will be subtracted from their energy expenditure (Actiheart^{TM}) rather than energy intake to avoid issues with participants' under-reporting food intake. We will induce this energy deficit by multiplying the weight of all individual foods from the 7-day diary by a factor gathered from the energy expenditure data, allowing us to determine the desired energy intake for the intervention period.

Intervention group 2

For the 4-week intervention period, as per the diet alone group, participants in intervention group 2 will reduce their habitual diet by 5000 kcal per week. However, there will be the addition of five, 30-minute aerobic exercise sessions per week. Each participant will be provided with a cycle ergometer which will be set up in the participants home, where all exercise training sessions will take place. Due to the progressive nature of the exercise increasing weekly, researchers will check-in with participants weekly via video/telephone call to ensure there are no issues. Participants will also be required to wear a HR monitor for at least 1 exercise session a week for us to determine that the intensity is increasing weekly.

Exercise sessions will be based upon individuals' perception of intensity using the 6-20 rating of perceived exertion (RPE) scale (where 6 = no exertion and 20 = maximal exertion). The target intensity based upon RPE will progressively increase throughout the duration of the training. During week 1 of intervention, the target intensity will be an RPE of 12 for 30 minutes, RPE of 13 for week 2, RPE of 14 for week 3 and RPE of 15 during week 4. These target intensities span the range from moderate-intensity exercise to the lower boundary of vigorous-intensity exercise.

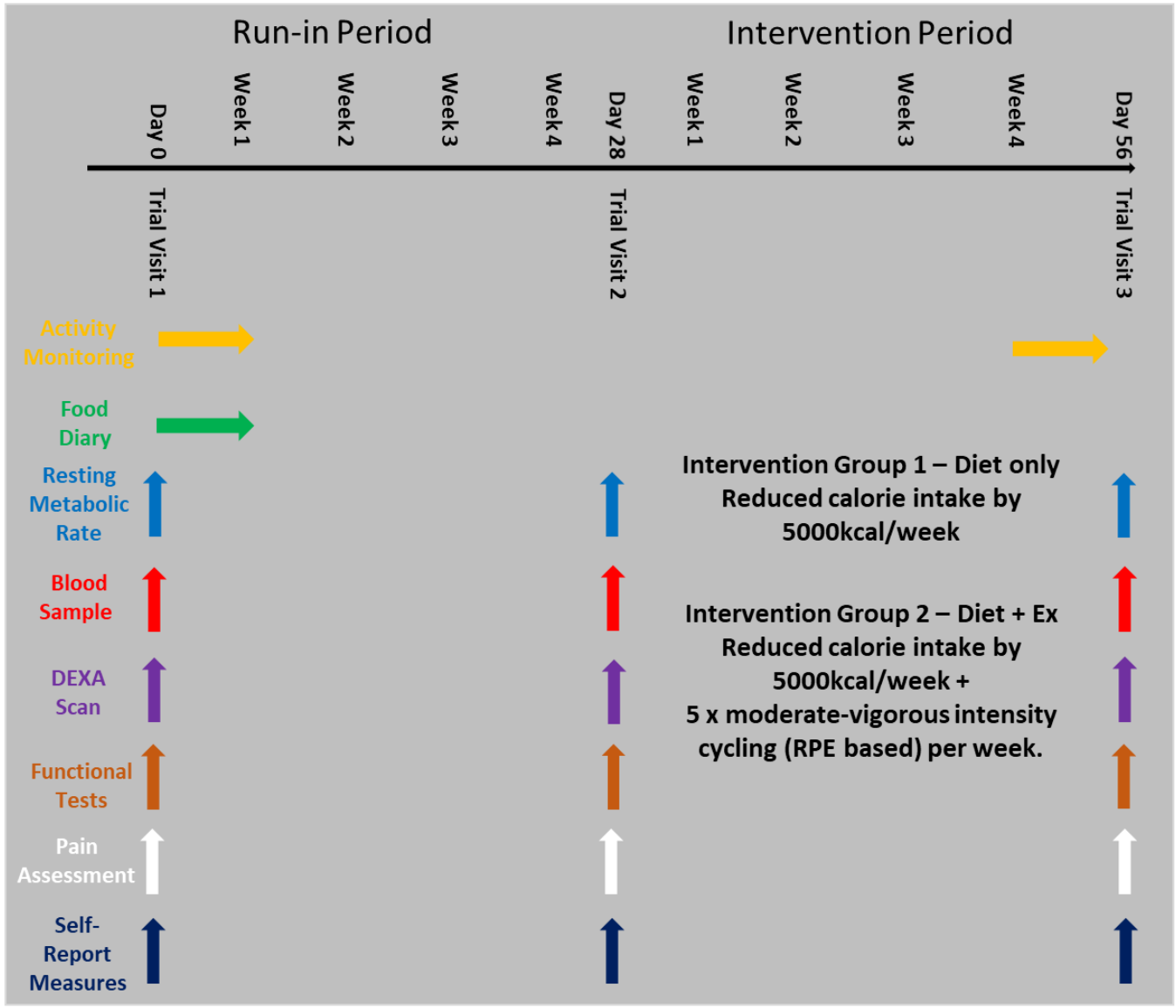

Figure 2. Detailed schematic of the two trial periods.

Procedures

Blood sampling

Blood samples of 20ml will be collected via venepuncture using local procedures, processed by centrifugation immediately (plasma samples) or left to clot for 30 minutes then processed (serum samples). In both cases, samples will be aliquoted into 500 microlitre cryovials and stored in ultralow temperature freezers at -80°C until use. Trial specific Instructions will cover laboratory processing of samples. Measurements of biomarkers will be by established laboratory assays, either immunoassay or electrochemiluminescence (Mesoscale Discovery), previously validated for use with plasma or serum, Watt Laboratory, Oxford; with CRP, glucose, NEFA and total/HDL cholesterol analysed using a clinical chemistry analyser at the Human Physiology laboratories at the University of Bath.

Lifestyle intervention

Diet

Based on their habitual dietary choices, participants' calorie intake will be reduced by 5000kcal.week^{-1} relative to measured energy expenditure during the week. The dietary restriction will be based on an absolute energy deficit achieved by the intake of smaller food portions.

Exercise

Participants will take part in 5 moderate-vigorous intensity (based upon RPE) stationary cycling exercise sessions per week for 30 minutes. This is in line with weekly recommendations of exercise for adults.

Disadvantages/risk and discomfort

Participants will be asked to give up their time, for pre-screening preliminary testing and 3 laboratory visits at the University of Bath. This might pose an inconvenience for some people.

Venous blood samples will be collected by venepuncture which can cause minor discomfort during the procedure and may result in minor bruising. In rare cases, there is the chance of

infection, though the occurrence of such events are further minimised by our strict institutional adherence to best practice and standard operating procedures.

Assessment and clearance for submaximal exercise will be determined by Physiotherapists employed by Virgin Care as part of the participant identification process. This will reduce the risk of adverse events during exercise testing. Based on participants' choice, exercise sessions will be subsequently conducted at the laboratories at the University of Bath or at home with equipment provided by the researchers – this option will be subject to the participants confirming that an additional individual will be available to be present during home-based training.

Symptoms in response to the prescribed exercise will be monitored throughout the duration of the intervention. Patients will be asked to rate pain in response to exercise using a 0-10 numerical rating scale. Scores greater than 6 during or after exercise will result in some reduction in intensity of exercise [35]. Additionally, should elevated joint pain persist for greater than 24 hours than the pre-exercise level, then the intensity of the subsequent session will be reduced. This will be continued until symptoms subside.

We will measure body composition and visceral adipose tissue in participants on the three trial days using a DEXA scanner. A DEXA scanner is a non-invasive radiographic technique that uses very low radiation (total protocol dose of 0.03 mSv) which is equivalent to experiencing a few days of average natural background radiation in the UK.

In line with current government guidelines and university policy surrounding COVID-19, strict social distancing measures will be in place when possible, and where not possible, suitable PPE will be provided for researchers and participants. If the participant or researcher present with any COVID-19 symptoms during the period of the research project, they will be required to quarantine in line with Government advice. Further, for the collection of expired gas during exercise, Douglas bags will be fitted with filters to prevent the potential spread of COVID-19. All lab spaces/research areas will be thoroughly cleaned and sanitized between each participant visit.

The above will be explained to the potential participant verbally and in the participant information sheet to ensure that they are fully informed before giving consent.

Methods

Blood storage

Serum blood samples will sit upright at room temperature for a minimum of 30 minutes and a maximum of 60 minutes before being centrifuged to allow a clot to form. Centrifugation will take place in a horizontal rotor for 20 minutes at 1800 g at room temperature. After centrifugation, we will pipette 500µl of the serum into cryovials which will then be stored at least -80°C in alarmed freezers. If samples are required to be moved after freezing, they will be shipped on dry ice to prevent thawing and re-freezing.

Functional tests

Participants will undertake the core set of functional tests recommended by OARSI:

- A 30-s chair stand test.
- A 40m fast paced walk test.
- A stair climb test (consisting of 11 stairs of 16cm height).
- Timed up and go test.

Self-report outcomes

As per recommendations[36], self-report measures will be completed after conducting of functional testing. The following measures will be obtained:

- The 42-item Knee Injury and Osteoarthritis Outcome Score (KOOS).
- The EQ-5D.
- Short Form Health Survey (SF-36).

Indirect Calorimetry

For the calculation of resting metabolic rate, indirect calorimetry will be undertaken. Participants will have expired air collected using Douglas bag system and analysed for fractions of expired oxygen and carbon dioxide, accounting for the inspired air composition[37] and utilising the equations of Frayn (1983)[38] and Jeukendrup and Wallis (2005)[39] for energy expenditure estimation.

Experimental pain measures

We will determine the mechanical detection threshold (MDT), pressure pain threshold (PPT) and dynamic mechanical allodynia (ALL) via the following methods:

- The mechanical detection threshold (MDT) will be measured with von Frey filaments that exert forces between 0.25 and 512mN onto the surface of the skin upon bending. The stimulus is applied using the method of limits. That is, the stimuli are presented from the lowest to the highest intensity. We will determine 5 thresholds, each with a series of ascending and descending stimulus intensities.

- Pressure pain threshold (PPT) will be measured using a handheld algometer. The stimuli will be applied to the skin at a rate of approximately 0.5 N per second. Participants will be instructed to verbally signal when the pressure turns to pain.

- Dynamic mechanical allodynia (ALL) will be measured using a brush as a light tactile stimulator acting as moving innocuous stimuli: exerting ~200-400nM of force. This will be applied with a single length stroke of ~2cm over the skin.

Storage and management of data and confidential information

Blood samples will be partially processed/analysed on trial days under an anonymised participant identification number and then kept locked away in the laboratory, at -80°C, which will only be accessible to the researchers involved. After analysis, samples will be stored until the work has been published. Any published work from the study will include no identifiable participant information. Participant information and results from the study will be stored in an Excel data sheet, located, and maintained on a university computer, locked with a password shared only between the research team.

Rewards and feedback

As part of the intervention period, if allocated to the exercise group, participants will be given a cycle ergometer for use in their own home for the duration of the study period. Individuals in the diet only group will be offered a cycle ergometer for one month and an initial supervised exercise session after the completion of the study.

The two intervention arms are designed to produce health benefits to the participants, including improved aerobic capacity and potentially reduced knee pain and increased knee function as a result of the exercise intervention. Individuals in the diet only group would be expected to lose a small amount of body mass. Individualised feedback documents will be given to all participants, which will highlight improvements that could made to their daily routines i.e., diet and exercise. This document will also include information gathered from all tests i.e., body composition, fitness level and blood tests.

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