Home Office

NON-TECHNICAL SUMMARY

Understanding musculoskeletal ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

bone, energy metaboilism, musculoskeletal ageing, osteoarthritis, osteoporosis

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult, Aged animal

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand the mechanisms underlying musculoskeletal health, and ageing.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Musculoskeletal disorders, comprising more than 150 conditions, are at the forefront of age-related conditions affecting approximately 1.71 billion people worldwide. The World Health Organisation has described them as ‘leading contributor to disability worldwide’ giving rise to enormous healthcare expenditures and loss of work. Development, growth, and maintenance of the skeleton are under complex biomechanical, endocrine, and genetic control and depend on integration of cellular events within the skeleton. When this regulation is disrupted, bone and joint diseases including osteoporosis and osteoarthritis, prevail, and this is very common in elderly adults.

Osteoarthritis is one of the most frequent age-related chronic musculoskeletal diseases. The World Health Organisation estimates that 10% of men and 18% of women aged over 60 have symptomatic osteoarthritis. Further, 40% of woman over 50 years are estimated to have an osteoporotic fracture.

Understanding the mechanisms underlying musculoskeletal disorders will provide invaluable insights and will have broad translational potential. It will, therefore, likely lead to long-term patient benefit and societal impact from a contribution to global economic activity. The clinical benefits are potentially huge and their healthcare, financial and societal impact is only set to rise in the ageing population.

What outputs do you think you will see at the end of this project?

It is anticipated that the outputs generated from this project will contribute and complement ongoing research into musculoskeletal health. The majority of the UK population will be affected by a musculoskeletal or joint disease as they age. These may include bone loss and fractures characteristic of osteoporosis, osteoarthritis or even cartilage to bone conversion such as in dyschondroplasia (a disease that affects the development and growth of bones and cartilage, a type of connective tissue, that can tolerate mechanical stress and acts as a supporting structure in the body). The proposed studies may therefore provide invaluable new knowledge of the mechanisms, specific target molecules, genetic predisposition which lead to these diseases, and ultimately offer new therapeutic approaches. Further, it will generate outputs in the form of: (i) peer reviewed publications (expected >10) (ii) invited seminars (iii) oral and poster presentations at scientific conferences and lay audiences.

Who or what will benefit from these outputs, and how?

The proposed studies are anticipated to provide new knowledge of the target mechanisms, molecules, and genes which lead to these musculoskeletal diseases, and ultimately offer novel approaches for treatments. Together, these will benefit immediately the scientific community. The immediate beneficiaries of this work will comprise academic researchers in multiple biological disciplines including, but not limited to bone and cartilage biology, osteoporosis, osteoarthritis, rare bone diseases, and joint imaging. This research will, therefore, benefit a wide range of researchers across the globe, with whom future collaborations can potentially be formed. These partnerships will encourage and facilitate interdisciplinary research and all parties would benefit from this. In the long-term, beyond the 5-year duration of the programme of work, this fundamental research will be extended, refined and may lead to drug targets and development of targeted successful treatments for many musculoskeletal diseases which will ultimately benefit members of the public with these debilitating diseases.

How will you look to maximise the outputs of this work?

I work closely with national and international colleagues where group efforts are made to understand the biological mechanisms underpinning musculoskeletal disease and ageing, and I will continue to nurture these productive collaborations for this work, as well as look to foster new ones where appropriate. I always strive to maximise the outputs of my research and will continue to do so for this work. I am fully committed to ensuring that any novel results generated are disseminated as broadly as possible across biomedical academic communities and that data are available for future interrogation. Similarly, I will always look to publish unsuccessful approaches as I believe these are just as important in our pursuit of understanding, and within the musculoskeletal field, journals often have special issues focused on these studies. Through the work generated by this project, I will also continue to play an active role in the public understanding of science relating to musculoskeletal, bone and joint diseases, and methods for prevention and management. My research work has featured at various Science Festivals. I have regular community meetings with representatives of elderly adult groups at the largest national charity for elderly people in the UK to increase awareness and understanding on how the research evidence is used to have an impact in society.

Species and numbers of animals expected to be used

- Mice: 3000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We will use wild type and genetically modified mice and models for achieving the aims of this project. Mice are required to enable us to examine the 'whole joint' rather than the separate components of the joint. We will breed genetically modified mice and use juvenile, adult and aged mice in our

experiments. This is because we are interested in understanding musculoskeletal health throughout the life course and, with ageing to improve it across the life course and reduce the likelihood of age-related musculoskeletal diseases.

Typically, what will be done to an animal used in your project?

Typically, a mouse will undergo induction of a skeletal pathology such as osteoarthritis either by surgical intervention (destabilisation of medical meniscus; a crescent-shaped band of cartilage in the knee that stabilises the knee and absorbs shock) or non-invasive skeletal loading (application of longitudinal compression to the skeleton) using axial mechanical loading machine to induce post-traumatic secondary osteoarthritis. Before or after this, animals may receive an injection of a compound by routes such as oral, subcutaneous, intravenous, intraperitoneal, or intramuscular to examine whether this protects against the skeletal pathology.

Mice will undergo either ovariectomy or castration to induce post-menopausal/andropausal osteoporosis. Mice that will have surgery will receive appropriate levels of analgesia for pain relief prior to surgery and additional analgesia to stop post-surgical pain.

Mice will have food restriction prior to mechanical loading of knee joints and reintroduction of the food. Fasting may be performed on more than one occasion e.g. over a two week period animals will be fasted every second day and knee joint loading performed on fasted or fed animals under anaesthesia. Mice (male and female, young, adult or aged) will receive no more than two regulated procedures, and experiments will last no longer than 8 weeks post-disease induction.

What are the expected impacts and/or adverse effects for the animals during your project?

The majority of the protocols in this project will not result in any adverse effects. Some mice will have surgery and, therefore, there is a risk of infection, although we will take every precaution to ensure this does not happen. Our use of surgical approaches will be kept to a minimum and pain relief during our protocols will be achieved through appropriate levels of analgesia peri-operatively and additional analgesia to stop post-surgical pain. Axial mechanical loads applied to mouse knee joints will induce strains in the higher range of those experienced physiologically equivalent to strenuous or high-impact activity. Soft tissue damage during application of the loading will be avoided by using polished metal or padded supports as appropriate. There have been no reports to date that describe compromised limb function and lameness in this loading model. Analgesia is not considered necessary after loading. We do not expect to see any adverse effects during fasting of animals. Drugs will be administered at non-toxic dosages and if unknown, this will be carefully tested.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

It is anticipated that the highest severity that an experimental mouse will experience in this PPL is moderate such as the ones undergoing surgery. The majority of animals (approximately 60%) will experience a mild severity.

What will happen to animals used in this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The aim of this application requires physiological context, and, therefore, this project will largely adopt an *in vivo* approach with mice as experimental model organisms. Use of mice is required to study the systemic changes, so they match as precisely as possible the circumstances in humans. Further, age-related joint deterioration is a 'whole organ' event, and studies that may use models of the joint's constituent components separately would therefore provide insight into only some aspects of the joint function.

Similarly, removing the activity of a gene provides information about what that gene normally does in a physiological (whole body) context and this information cannot be obtained from human studies or cell culture models where, for example, cell-cell interactions and whole body regulatory pathways are lost. Also, the genetic tools and models required for the studies detailed herein are readily available in this species.

Which non-animal alternatives did you consider for use in this project?

In vitro ('test-tube') and cell culture-based alternatives have been, and will be, invaluable to biomedical research and I will always consider these in my experimental design wherever possible. I have fully acknowledged their strengths, reviewed their use for others, but I am aware and appreciate their limitations, as detailed below.

Why were they not suitable?

In vitro approaches along with in silico computational models have a number of recognised limitations. Ultimately, they fall short in providing the integrated, organ-level, physiologically intact environment that animal models provide and, thus, make interpretation of indirect effects of agents on bone and other tissues of the musculoskeletal system impossible to detect. These *in vitro* approaches also fail to produce range of structural abnormalities in joint architecture that can be seen, and do not represent all *in vivo* tissues.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers of animals are reviewed and based on my previous work. This was used to estimate the minimum number of rodents required for achieving statistically significant differences between groups in each study.

Once we have some preliminary/pilot data, we will look to perform power calculations to determine group sizes for future studies. In-house discussions with a biostatistician will also be held to review our experimental designs.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will always aim to reduce the numbers of animals we use, and routinely use the NC3Rs' (National Centre for the Replacement, Refinement and Reduction of Animals in Research) Experimental Design Assistant and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines. Power analyses are applied to identify the minimum number of experimental animals needed to answer the scientific question being posed in each study. For example, we have established that in our surgical model of osteoarthritis, a minimum of 6 mice in each group is required to have statistically significant differences. Wherever it is possible we will exploit contra-lateral limbs as internal controls to reduce the number of animals required.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Breeding will be performed in a way to maximise efficiency, reduce surplus mice and utilise both sexes in the experiments. Smaller group size pilot studies will always be conducted where appropriate. In our studies, we will optimise the experimental output where possible, thereby, enabling serial data acquisition and removing the need for the humane killing of multiple groups of mice at set time-points. This will be further advantaged by our current attempts to acquire funding for an *in vivo* computed tomography scanner which allows imaging of bone microarchitecture without the need for killing mice.

All experiments using live animals will adhere to The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines on full and transparent reporting. Good principles of experimental design will be applied to ensure sufficient group sizes are used to adequately test the hypotheses. Sample sizes are estimated from pilot studies and previous data using power analysis. Where possible surplus tissues may be made available to other research groups.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Genetically altered mice bred and maintained are of non-harmful phenotype. Substances are prepared and administered using sterile techniques. The route of administration is via the least invasive method appropriate to the model. The volume of substances to be used, preparing for and undertaking aseptic surgery, and other aseptic techniques will be in accordance with the NC3Rs on refinement and the Laboratory Animal Science Association (LASA) good practice principles and guidelines.

Loading forces will be applied non-invasively using a computer controlled loading device, where loads will be applied to the joints. Drugs will be administered at non-toxic dosages and if unknown, this will be carefully tested. Where surgically approaches are necessary, analgesia will be provided to minimise discomfort following veterinary advice.

Why can’t you use animals that are less sentient?

Here, we will use mice as they are the most suited to be able to answer the biological questions which we pose in each study. The genetic tools and models required for achieving the aims of this project are readily available in this species, the skeletal system of the mouse is similar to human hence it is representative and translatable. Further, mice are required to enable us to examine the 'whole joint' rather than the separate components of the joint, and to take into consideration the whole physiology of the musculoskeletal system. This project will use juvenile, adult and aged mice because we are interested in understanding musculoskeletal health across the life course and in particular, with ageing, and hence more immature life stages cannot be used.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The procedures that are in place to administer substances and monitor the animals are 'fluid' whereby any opportunity to refine a technique or ensure additional monitoring is performed where necessary, which is carried out by the animal technicians and licensed scientists. Refinements to minimise the harms and help to support the animals include acclimatisation to studies, tunnel/cup handling and single needle use. Pain management during our protocols will be achieved through appropriate levels of analgesia peri- and post-operatively. Soft food will be provided to the animals post surgery.

Any relevant refinements made including following surgery are discussed and disseminated to the other users by the animal care scientists. The veterinary surgeon will also offer suggestions for refinements where necessary.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the guidelines published by The ARRIVE. We will also consult the published LASA guidelines on aseptic surgery, guiding principles on good practice and the 3Rs website (www.nc3rs.org.uk).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will maintain close interactions with the relevant Welfare, Training and Named Information Officers. The PPL holder will stay informed of advances in the 3Rs by regularly checking the NC3Rs webpages (https://nc3rs.org.uk/the-3rs) and the newsletters circulated by the Named Information Officer. Moreover, the PPL holder will join the University of Cambridge internal 3Rs mailing list and attend appropriate seminars, symposiums and conferences deemed suitable.