Home Office

NON-TECHNICAL SUMMARY

Understanding the role of systemic inflammation in cardiovascular disease and obesity

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

Key words

Cardiovascular, Obesity, High blood pressure, Diabetes, Therapy

Animal types	Life stages
Mice	juvenile, adult, neonate, embryo, pregnant

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?

This project aims to understand how increased fat in obesity causes cardiovascular diseases such as high blood pressure and diabetes, and develop new ways to treat these diseases.

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?

Cardiovascular disease is the most common cause of death in the world, and one of the most common causes of disability. Obesity is very common, and it is one of the biggest causes of cardiovascular disease. However it is not easy to lose weight by dieting, and most people will put back on the weight they lose on a diet within two years. Treatments for the diseases caused by obesity are not very effective, and many of them have unpleasant side effects which leads to people not taking their medicine. Therefore, we need to understand how obesity causes disease, so that we can develop better treatments.

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

These studies will generate new data to advance scientific knowledge of the development of cardiovascular diseases in obesity. These data which will be published and presented to the cardiovascular science community. This knowledge may identify new approaches to treatment of obesity-related diseases, and new drug targets. In addition, these data may refine animal models used by others.

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

These studies will further the knowledge of the research group, and our future research will build on these findings. Ultimately, our findings may highlight new targets from drug development for pharmaceutical companies or new approaches to treatment of obese patients by clinicians; thus translating into patient benefits.

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

Data will be published in open-access journals and presented at scientific meetings. Where feasible, data and resources will be shared with other researchers and collaborators.

Species and numbers of animals expected to be used

- Mice: 2440

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.

The structure and function and the mouse cardiovascular system closely models that of a human. The majority of our experiments will be conducted on mice that are around 4 months old; when they are full grown. This is because we are interested in the effects of obesity, therefore many of our studies will involve using mice fed a modified diet to mimic obesity, and we need to allow time for mice to feed and grow. Most animals used under this licence will be purchased from appropriate suppliers, however for some experiments we will breed our own mice that have been genetically modified in a way that will help us study diseases. Breeding will involve the use of neonates/embryos, adults, pregnant adults and juveniles.

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

The majority of mice in this project licence will undergo mild, non-invasive or minimally invasive tests on their cardiovascular systems very similar to those done in humans, for example blood pressure, recordings of electrical activity in the heart, or blood tests. These may involve gently restraining the animals, and may be done multiple times. For some experiments, like echocardiography, we will need to anaesthetise the animal to be able to take for these recordings.

To study how obesity causes diseases, mice may be fed a modified diet such as a high fat diet, usually for around 3 months. We will also investigate how exercise in obesity might treat diseases. For these experiments, some mice will be trained to exercise either on a treadmill or by swimming, usually for around 6 weeks.

For some experiments, we will administer by injection or oral gavage substances such as potential new treatment or drugs which mimic disease, so that we can study how they work. This is usually daily for a short period of time e.g. 5 days.

Some animals will be infected with a parasitic worm.

In a small number of mice (~7%), a device that can record blood pressure and/or recordings of electrical rhythm in the heart will be surgically implanted under the skin.

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

For some experiments animals may need to be gently restrained or anaesthetised, which may cause mild stress but not for long and the animals will recover almost immediately.

For mice fed a modified diet, we will ensure that diets are palatable and all of their nutritional needs are met. Sometimes these diets may make their fur greasy, but this won't cause any lasting harm. These diets may cause the animal to become obese and have high blood pressure or diabetes. In our experience, the obesity has no effects on their ability to move around and they don't exhibit any symptoms of illness, so although they are fat they appear to be normal and will not suffer.

Some studies have suggested that exercise can cause stress in mice. In our experience, we have never seen any signs of stress in our models. Furthermore, we have measured stress hormones in their blood and found they are normal. Mice will not be exercised to exhaustion.

Animals receiving injections or having blood taken will experience mild, transient pain, but no lasting harm.

Some animals will be infected with a parasitic worm, these worms are not harmful and do not normally result in any suffering or distress.

In the small number of mice (~7%) undergoing surgery they will experience some discomfort for a few days after surgery and some mild to moderate pain. Also, they may lose weight in the first few days but they should make a full recovery.

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)?

10% of mice will experience moderate severity.

59% of mice will experience mild severity.

31% of mice will experience sub-threshold severity.

What will happen to animals at the end of 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?

We wish to study how obesity causes cardiovascular diseases. Cardiovascular diseases involve multiple organs that interact with each other. Specifically, we are interested in how fat causes these cardiovascular diseases, which is widespread around the body. Therefore, because of the complex and widespread nature of these diseases, to be able to study them we need an intact organism with a similar cardiovascular system to humans.

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

We have considered and researched the literature on organ-on-a-chip technology, cell culture and computer models.

We cannot consider non-protected species such as fruit flies, as they do not have a comparable cardiovascular system to humans for study. Fruit flies may be useful in studying genetic causes of cardiovascular disease, however we are studying the effects of lifestyle (obesity) on cardiovascular disease, and this could not be replicated in a fruit fly.

Why were they not suitable?

There are no existing computer models of blood vessels that include modelling the effect of fat on the vessels, which is key to our studies. State of the art organ on a chip technologies cannot yet replicate the contraction and relaxation of blood vessels, which is important to studying how blood pressure becomes increased in obesity. The same applies to cell culture. None of these methods mimic the complex nature of interactions between multiple organ in the body. As we wish to study the onset and progression of disease in obesity, we need an intact, live system. Prior to performing studies in live animals, we use tissue taken from culled animals to conduct experiments outside of the body to direct our research. Specifically, we can dissect out arteries with their surrounding fat intact and measure their contraction and relaxation in an organ bath. We can apply drugs and examine their effects on the vessel function, and promising drugs may be progressed to experimentation in live animals.

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?

The number of animals needed for each experiment is calculated based on our previous experience and experiments, and will depend on the variable being examined. Using the typical amount of variation in our earlier experiments allows us to calculate the minimum numbers of animals used whilst ensuring that the results are statistically meaningful. For example, when looking at the differences in blood pressure between two types of mice, we have calculated that each group will need 24 mice.

Also, we have used our previous data from our annual returns of procedures to estimate the total numbers.

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

We have used resources from the NC3Rs and Royal Veterinary College to help with our experimental design, and consulted with a statistician. To reduce numbers, it is important to reduce variability. To reduce variability, we will do the following:

- Where possible the same person will conduct the experiments, and information regarding which animals are in what treatment group will be hidden to avoid bias.
- A standard operating procedure will be followed for experiments and analysis
- We will use the same ages of mice
- Experiments will be conducted at the same time of day
- Animals will be randomly assigned to groups
- A randomised block design will be used

In addition, we will follow a sequential design that includes an interim review after we reach the half way point i.e. 12 animals per group. If at this time point the evidence is convincing, we can terminate the experiment and thus reduce the number of animals. This strategy has the potential to reduce the number of animals required by 15-25%.

There are important differences in male vs female biology that would significantly increase variability in our studies, therefore if we were to combine the use of male and female mice, this would greatly increase the number of mice needed. To avoid this, most of our studies will be performed in male animals. Experiments comparing the differences in female vs male biology will begin on a smaller scale and results will be used to direct future studies.

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

At the end of the experiment, we will harvest as many tissues as possible. Tissues we don't use immediately will be frozen and stored for use later, or for sharing with other researchers.

When starting new studies where the effects of administering a substance are unknown, we will start by testing in a small pilot study group.

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.

The majority of mice in this PPL will undergo minimally or non-invasive tests on their cardiovascular systems very similar to those done in humans, for example blood pressure, recording of heart electrical activity or blood tests, enabling us to precisely measure their cardiovascular health with little to no pain, suffering, distress or lasting harm. These may involved gently restraining the animals, and some mild discomfort following blood withdrawal. We will follow NC3Rs guidelines on blood collection techniques and volumes to ensure harm is minimised. For some experiments, like echocardiography (ultrasound scans of the heart), we will need to anaesthetise the animal to be able to take for these recordings. This won't be for long and animals will recover quickly. Performing these tests will allow us to study the progression of disease, much like we would in a human.

To study how obesity causes diseases, mice may be fed a modified diet such as a high fat diet, but this will not cause any harm or distress. We will ensure that diets are palatable and all of their nutritional needs are met.

We will also investigate how exercise in obesity might treat diseases. For these experiments, some mice will be trained to exercise either on a treadmill or by swimming. To minimise stress and to help the animal acclimatise, the amount of exercise will be gradually increased. These approaches allow us to closely mimic human behaviour and the most popular exercises used by the general public, and they will not cause any lasting harm.

For some experiments, we will administer substances by injection or by oral gavage. This will allow us to investigate chemicals that may be important in the development or treatment of disease. Animals will experience mild, transient pain or discomfort, but no lasting harm. We will ensure we use the most appropriate and minimally invasive method, therefore causing the least amount of pain.

Some animals will be infected with a parasitic worm. However, the worms we use are not harmful, and do not normally result in any pain, suffering, distress or lasting harm.

In a small number of mice (~7%), a device that can record blood pressure and/or electrical activity in the heart will be surgically implanted. These animals will experience some discomfort for a few days after surgery and some mild to moderate pain which will be treated with pain killers, but they will make a full recovery. This is sometimes needed to allow us to take these measurements in live, unrestrained animals.

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

Non-mammalian animals don't replicate the human cardiovascular and/or immune system to provide relevant results. As we study obesity, many of our mice will need to be fed a modified diet to induce obesity, and this will take time. Comparisons must always be made in the same age animals to reduce variation. Measures need to be taken throughout the animals life to track disease progression, therefore cannot be conducted under terminal anaesthesia.

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

Welfare of the animals will be monitored by trained technicians and researchers. A scoring system will be used to help identify any pain or suffering early and take appropriate measures such as administering pain killers. The scoring system will monitor changes such as weight, temperature, appearance, and heart rate. If these effects are seen and either cannot be treated or do not resolve within a day, or if severe effects are seen, the animals will be humanely killed.

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

We will make use of NC3Rs resources, PREPARE guidelines, LASA principles, and published guidelines such as the report by the Joint Working Group on Refinement on substance administration [1].

1. Morton DB, Jennings M, Buckwell A, et al. Refining procedures for the administration of substances. Laboratory Animals. 2001;35(1):1-41.

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

We have signed up for the NC3Rs newsletter, and will regularly check information on the website and attend relevant symposia and events where possible both externally and internally within our Institution.