



Home Office

NON-TECHNICAL SUMMARY

Understanding the regulation of fat mass and its association with metabolic disease.

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

Key words

Metabolism, Adipose Tissue, Obesity, Type 2 diabetes, Insulin sensitivity

Animal types

Life stages

Mice

adult, neonate, juvenile, pregnant, embryo

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?

To define the biological function of genes linked to human fat mass regulation and metabolic disease and in some instances, leading to the identification of novel therapeutic drug targets.

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?

Both too much (obesity) and too little (lipodystrophy) fat are strongly associated with human metabolic disease e.g. type 2 diabetes and non-alcoholic fatty liver disease (NAFLD). Considerable progress has been made in understanding the genetic factors which cause obesity and lipodystrophy in humans but in many cases the function of the relevant genes remains unclear. Mice have proven to be very valuable animals for advancing understanding of the biological function of the genes identified. Ultimately this work has and may yet lead to the discovery of novel therapeutic targets for human metabolic disease.

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

1. We will characterise the body weight/ fat mass and fat distribution of genetically altered mice. These mice will be designed to provide novel information on genes implicated in human obesity.
2. We will then document changes in weight and fat mass of these mice in response to a regular or high fat diet in an attempt to assess their capacity to adapt to nutritional challenges.
3. In cases where the genetic changes do alter weight and/or fat mass, we will also document the impact of these changes on carbohydrate/ fat metabolism and on cold temperature tolerance, providing new information on how the genes under investigation impact human metabolism and health.

This work will be presented at major scientific meetings around the world and will be published in scientific journals.

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

The annual UK-wide NHS costs attributable to overweight and obesity are proposed to reach £9.7 billion by 2050. Most of this cost relates to dealing with the metabolic diseases associated with obesity; including type 2 diabetes, fatty liver disease and cardiovascular disease. Interestingly, lipodystrophies, which are diseases characterised by a pathological lack of body fat, are also associated with these metabolic diseases. So both too much and too little fat can lead to serious metabolic disease but why this is so, remains incompletely understood.

The generation and characterisation of mice in which the expression of genes we know are linked to human metabolic disease through an effect on fat mass will help us to generate new insights into the

causes of type 2 diabetes and other common metabolic diseases, and to potentially reveal novel therapeutic drug targets.

In the short term, the scientific community will gain new information on the genes which we know will be of interest as they will be selected based on being associated with human weight and fat mass.

In some instances, the molecules under investigation may themselves be treatment targets or indirectly inform new treatment strategies for common metabolic problems such as obesity, type 2 diabetes and fatty liver disease.

The knowledge we generate may also help patients with rare but very serious metabolic problems such as lipodystrophy.

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

We already collaborate with several other groups studying metabolic disease around the world, and will publish our results in scientific open access journals. We will also present the findings at scientific meetings as posters and oral presentations. This typically applies to positive and negative findings as all our work relates to novel genetic models so we simply describe what we observe either way - it is not really 'positive' or 'negative' in the same way that a trial of a new treatment would be.

Species and numbers of animals expected to be used

- Mice: 8470

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 purpose of this project is to understand the biological function of genes linked to human fat mass regulation and metabolic disease. Mice have been used for decades to understand how genetic alterations cause obesity and why genetically altered mice prone to obesity may develop type 2 diabetes, so are well-established animals for this type of project. Insulin action is a key factor in the link between obesity and diabetes. Its complex actions on several different organs requires whole animals in order to study how all the different organs and systems in the body work together.

The project is focussed on changes in fat mass and metabolism which typically only manifest after weaning in mice so we will focus on juveniles and adult mice.

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

Firstly we will use established standardised breeding protocols to generate genetically altered mice.

These mice would then be group housed in their home cages while being supplied with a regular diet or a high fat diet for 24 weeks. Occasionally mice may be singly housed (for up to 48 hours) in order for us to accurately determine how much food a single mouse is eating or to determine its metabolic rate. We might also singly house mice (for up to 7 days) whilst reducing their usual calorie intake by up to 30%. Alternatively we might undertake a similar calorie restriction for a more prolonged period (up to 3 months) in group housed mice. During this time they would be weighed on a weekly basis and blood samples would be taken to check glucose and insulin levels no more than 4 weekly, unless a mouse showed signs of diabetes (drinking excessively/ passing excess urine). We would also assess fat mass regularly using time domain nuclear magnetic resonance (TD-NMR), which is a piece of equipment used to measure body fat in the awake mouse. Then towards the end of the study we might perform a glucose tolerance test using an intraperitoneal (this means into the abdominal cavity) glucose injection. After recovery for one week, the mouse might then have an insulin tolerance test (intraperitoneal injection again) before being humanely killed for tissue collection and final blood collection. Before the glucose and insulin tolerance tests mice will be fasted for up to 6 hours.

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

Our studies will involve the following:

1. Blood sampling with its associated handling stress and transient discomfort.
2. Intraperitoneal injections with their associated handling stress and transient discomfort.
3. Some mice may develop diabetes which will result in increased drinking and urine production. If this is noted we will monitor blood sugar levels and if they go too high the animal will be killed.

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)?

Mice:

Mild: over 90%

Moderate: less than 10%

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 need to use animals as diseases like diabetes involve interactions between several key organs like the fat, liver and muscle. Many of the questions we are interested in can only be studied in complex organisms like mice, rather than simpler organisms like flies, worms or fish which don't have fat under their skin as humans and other mammals do. Where possible we do and will study more basic questions in cells, yeast or flies, but none of these has true fat tissue so they are inadequate to really address the role of the genes/proteins we are interested in.

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

We perform some studies using tissues removed from humanely killed mice, such as using adipose (fat) tissue explant (tissue removed from a dead mouse and then maintained in a fluid bath) assays that allow us to analyse some of the tissue functions whilst minimising suffering of the mice.

We also use primary cells isolated from the mice, like Mouse Embryonic Fibroblasts (MEFs) or Ear Mesenchymal Stem Cells (EMSCs), or well established cell lines to perform experiments in cells grown in the laboratory. We have used MEFs or genetically engineered cells, to enable us to work with cell lines representative of the mouse strains, thereby reducing animal numbers.

Why were they not suitable?

For metabolic studies we need to be able to use a system which involves interactions between different organs; specifically fat, liver and muscle as these are all key organs in insulin action, and impaired insulin action is a major factor in causing type 2 diabetes. Cells and animals, such as insects and worms, do not have body systems that work in the same way they do in humans and mice.

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?

We have conducted very similar studies to those proposed herein in the past, so have based our current estimates on this prior experience.

We also work in an institute focused on understanding metabolism so have several colleagues with overlapping interests. Our institute also has a core metabolic phenotyping team who ensure that expertise is shared across the institute and provide support and advice to each team. So again relevant shared experience has been used to estimate our numbers.

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

A key aspect of reduction involves studying only carefully selected strains of mice. My research programme does not involve large-scale use of genetically altered mice but instead focuses on very specific questions which cannot be addressed in humans or other simpler organisms.

The methods we use to characterise mice have been used extensively within my group so we have the necessary expertise to work out the minimum number of mice needed to answer the questions we are working on. Typically we would assess a new mouse strain in a group size of 8-10 mice on a basic protocol.

Non-invasive technology: Use of sequential non-invasive methods for phenotyping means that we can generate data without killing the mice, thus minimizing the number of mice needed.

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

Breeding: This will be done by experienced staff who work for the core facility in our institute. We will also obtain animals from relevant suppliers where these exist rather than generating lines ourselves, thus minimizing breeding.

Our work is guided by the NC3R's PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence), the ARRIVE (Animal Research: Reporting of In Vivo Experiment) guidelines, and the UK Home Office "Assessment Framework" on the efficient breeding of genetically altered animals (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf).

We write comprehensive Study Plans for each experiment which include:

- 1) a statement of the experiment objective(s)
- 2) a description of the experiments, covering topics such as proposed diets, the size of the experiment (number of groups, number of animals in each group), and the experimental materials to be used
- 3) an outline of the method of analysis of the results is included (which may include the planned statistical approach).

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.

We will only be using mice where information from human studies has not already led to comprehensive understanding of the function or impact of a gene or gene variant, and where a simpler cell based or model organism cannot be used.

Genetically altered/modified mice that develop obesity and diabetes have proven for decades to be valuable in helping us in this type of research and so are a particularly suitable animal for our experiments.

All of the genetically altered mice will be used in tried and tested procedures. We are not expecting the procedures (e.g. blood sampling and injecting drugs) and the majority of genetic alterations made to cause more than mild and transient discomfort which means our animals are expected to remain healthy as well.

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

Obesity typically causes diabetes by impairing insulin action. Insulin acts on several organs including fat, the liver and muscle so we need an organism with these organs and in which insulin action can be evaluated in each of these organs - this is not possible in simpler organisms. My laboratory has experience with cells, yeast and insects so is well placed to know when these can and cannot be used.

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

To improve the quality of life of the animals we:

- reduce stress by group housing where possible to keep singly housed mice to a minimum.
- use environmental enrichment (EE), within what is available to us at our animal facility. In general, EE is an animal housing technique composed of increased space, physical activity, and social interactions, which in turn increases sensory, mental, motor, and social stimulation. Igloos, running wheels, saucer wheels, fun tunnels, and other objects in the housing environment provide stimulation by promoting exploration and interaction. EE can be maintained when animals are handled (e.g. handling tunnels), thus minimizing stress when for example an injection is needed.
- use a series of non-invasive methods for characterising mice so that we can generate useful data without killing the animals, thus minimising the number of mice needed
- use pain killers to lessen pain.
- provide 'behavioural' training to mice undergoing specific procedures (e.g. acclimatization to single housing in cages used to measure food intake and energy burned).

- use scoring sheets to monitor the health of animals undergoing procedures.

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

Laboratory Animal Science Association (LASA) guiding principles documents of aseptic technique (https://www.lasa.co.uk/current_publications/)

ARRIVE (Animal Research: Reporting of In Vivo Experiment) guidelines for preparing papers for publication (<https://www.nc3rs.org.uk/arrive-guidelines>)

PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines for planning our experiments (15 topics including formulation of the study, dialogue between scientists and the animal facility, and methods) (<https://www.ncbi.nlm.nih.gov/pubmed/28771074>).

The UK Home Office "Assessment Framework" on the efficient breeding of genetically altered animals (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf).

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

To be informed about latest advances we will primarily use the National Centre for the Replacement and Reduction of Animals in Research (NC3R) website (<https://www.nc3rs.org.uk>). It provides an extensive library of 3Rs guidelines, resources, practical information and themed hubs. It also provides links to publications, other online resources, and video and training materials.

Implementation of the advances will be defined on a case-by-case basis, and will be informed by the latest NC3R recommendations.

We also share expertise across our institute with regular internal seminars and have an external seminar series so hear from other experts on a regular basis.

We have a team of researchers at our institute who specialise in using mice in obesity and diabetes research and they regularly provide us with updates on new advances.