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NON-TECHNICAL SUMMARY

Understanding how perturbing the function of fat tissue leads to 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

Metabolic disease, Adipose cells, Lipodystrophy, Obesity, Insulin resistance

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

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 understand how genes regulate fat tissue function and the risk of metabolic disease, with a view to identifying novel treatments for metabolic diseases such as type 2 diabetes and fatty liver disease.

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) body fat is strongly associated with human metabolic disease e.g. type 2 diabetes, fatty liver disease and cardiovascular disease. 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 and exactly why disturbing their action/s leads to metabolic disease remains unclear. Mice have proven to be very valuable animals for advancing understanding of the biological function of the genes and their links to disease. 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 deepen understanding of genes linked to human lipodystrophy, obesity or changes in fat distribution.

2) We will understand why adipocytes (fat cells) die in obese mice (and humans) triggering a chronic inflammatory response, thought to contribute to metabolic disease.

3) We will understand how calorie restriction (to approximately 30% of usual daily intake on a high fat diet i.e. in an obese mouse) so rapidly reverses insulin resistance.

This work will be presented at major scientific meetings around the world, will be published in scientific journals and may reveal new ways in which to improve treatments for people with metabolic disease.

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

Roughly 10% of the annual UK-wide NHS budget covers costs attributable to overweight and obesity. 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. Insulin resistance is strongly associated with both obesity and lipodystrophy and is thought to mediate the link between these problems and metabolic diseases.

The generation and characterisation of mice in which the expression of genes we know are linked to human insulin resistance and 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: 6500

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 deepen understanding of the genes which regulate fat mass and insulin action, and why altered function of these genes is associated with 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 either use established standardised breeding protocols to generate genetically altered mice, or we will generate tissue specific (usually fat tissue but potentially also other key metabolic tissue such as liver and muscle tissue occasionally) genetically altered mice. In order to generate genetically altered mice, specialised breeding procedures will be required in some instances. Some female mice (genetically altered or not) will be given a drug containing hormones that cause ovulation of an increased number of oocytes (eggs) than is typically observed in a natural ovulation. This is called superovulation and it will involve two injections, 48 hours apart, either into the body cavity (intraperitoneal) or under the skin (subcutaneous). For collection of oocytes, the animals will be humanely killed. If embryos are required, females will be mated first and at the desired developmental time point humanely killed and tissues collected.

Some female mice (genetically altered or not) will be mated with a sterile male to produce pseudo-pregnancy (i.e. making the uterus receptive for implantation of embryos) and then undergo either non-surgical or surgical embryo transfers. During non-surgical embryo transfer, embryos will be inserted directly into the uterus with a special device without any surgical intervention. Non-surgical embryo transfer will be used whenever possible as it will cause the least harm. In some cases, surgical transfer may be required, if for example there is persistent low efficiency of non-surgical transfers or if it is desirable to transfer embryos at very early stages. This will involve a surgical procedure to place the embryos in the uterus. If surgical transfers are required the animals will have surgery under anaesthetic and be provided with post-operative care, including pain relief, to minimise pain and distress. When the aim is to generate a new mouse colony from frozen sperm or embryos, live offspring will be born following embryo transfer. Animals that were used for non-surgical transfers can be re-used once again. These animals will be humanely killed either following weaning of live offspring, or at an appropriate developmental stage for post implantation embryo analysis.

Some male mice that have not been genetically altered will undergo surgery for vasectomy. They will have surgery under anaesthetic and be provided with post-operative care, including pain relief, to minimise pain and distress. These males will be used to mate with females to induce pseudo-pregnancy, (i.e. making the uterus receptive for implantation of embryos). These males may be kept alive to mate with other females. At the end of mating these animals will be humanely killed. Vasectomised males will be singly housed between matings.

In order to generate adipose specific tissue genetically modified mice, in some instances, a brief abdominal surgical procedure will be performed on mice, lasting approximately 10 to 20 minutes. This surgery is conducted in order to access the gonadal fat pads. Once exposed, these fat pads will be injected with specific reagents, typically AAVs (Adeno-Associated Viruses), to facilitate targeted gene modifications in the tissue. In order to modify gene expression in liver tissue, we may inject AAVs into the tail vein in 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 up to 24 weeks. Alternatively we may reduce usual calorie intake by up to 70% for up to 3 days. Alternatively we might undertake a more modest calorie restriction of up to 30% 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 from the tail vein 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 Echo Magnetic Resonance Imaging (Echo MRI), 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 typically be fasted for 6 hours but we may also fast mice overnight (12-16 hours) for some metabolic tests.

As fat tissue is particularly important in insulating animals, we may also want to study temperature regulation in some mice. To do this we may inject a small temperature probe under the skin of mice, and/or alter the temperature of the room in which the mice are kept to see how they tolerate colder or warmer temperatures. Most mice adapt readily to a broad range of temperatures and the mice would be monitored daily. In some mice, we will anaesthetise them before injecting norepeniphrine, which is a hormone whose level rises in temperatures in order to increase body heat, in order to see how the mice respond to this hormone.

In order to assess how mice use or store nutrients/ energy in key metabolic tissues such as fat and liver, we might cannulate a superficial/ deep vein (under terminal anaesthesia) infusing insulin, glucose, lipids or metabolic tracers. Blood samples may be taken before, during and after administration of substances, before the mice are humanely killed for tissue collection and final blood collection.

In some instances, mice might be implanted with a small pump device (underneath the skin or injected (intraperitoneally)) that facilitates slow release of agents such a drugs or metabolites. These animals can then be monitored to assess the impact of the agents on weight, insulin action and/or metabolic health.

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 or subcutaneous injections with their associated handling stress and transient discomfort.

3. Exposure to altered diets and/or short lived calorie restriction. High fat diets will be associated with greater weight gain and rarely diabetes (see point 5 below). Calorie restriction will be associated with weight loss. This will be within closely monitored parameters that take into account other important aspects of their appearance and behaviour.

4. Echo MRI in conscious mice is associated with a short period (less than 3 minutes) of stress related to the noise of the MRI machine and the need for restraint within a tube during the measurement.

5. 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.

6. A minority of animals will also undergo surgery that will require a general anaesthetic. Inevitably, as with any operation, animals will have some discomfort in the immediate hours after the operation at the site of the incision. However, this will be minimised by administration of painkillers under instruction from a veterinarian. The general anaesthetic needed for this surgery may also make the animals less active and less hungry in the first day after the operation but we expect them to recover their appetite and vitality within 48 hours.

7. Thermal adaptation challenges (exposure to cold or warm environments) are typically well tolerated, but in instances where mice display an abnormal reaction to this (usually seen within the first 6 hours), the animals will be returned to their normal housing environment and carefully monitored for 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)?

Subthreshold: 59%

Mild: 29%

Moderate: 12%

Severe: 0%

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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 and fatty liver disease 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 partially avoid use of live animals in some studies by using tissues removed from humanely killed mice, such as using adipose (fat) tissue explants (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 tissues alone cannot provide the metabolic fluxes of macronutrients and hormones which are so important in the control of metabolism 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?

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 (such as glucose and insulin tolerance tests) 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.

We increasingly study both male and female mice, as many studies have now shown that they do not always respond to metabolic perturbations in the same way. However, in instances where the responses between males and females are similar it means that fewer mice are needed in total.

Generating tissue specific genetically altered mice using injected AAVs means that far less breeding is required to generate experimental groups of mice of suitable sizes.

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).

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.

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).

Use of novel approaches to generate tissue specific (e.g. just in fat tissue or just in liver tissue) genetic changes using viral vectors which can be injected. This will reduce the number of mice usually needed to generate a tissue specific knockout model.

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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, fatty liver 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 (e.g. glucose or insulin tolerance tests, high fat diet feeding, overnight fasting). 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. Immature life stages are not suitable for the study of insulin action which is most relevant to animals feeding on regular diets.

We do undertake some studies in terminally anaesthetised mice (e.g. insulin clamp studies), but terminal anaesthesia does obviously prevent exercise which has an important impact on glucose metabolism, so in many of our studies we do need to study awake animals.

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.

- 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 and provide post-surgical care such as hydrogel, mash and alternative soft-cushioned bedding within home cages.

- provide 'behavioural' training to mice undergoing specific procedures (e.g. acclimatization to handling needed for injections).

- 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.

Further supplemental information and guidelines are provided through the Named Information Coordinator (NIO) within the university. The 3R's enquiry list and the internal colony management meeting are also addtional resources through which we have been able to identify useful advances, share animal strains and seek expertise in particularly animal techniques.

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

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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. Furthemore, our institution has a regular advisory board meeting to facilitate meaningful dialogue across the license holders, researchers and technicians. Through these meetings we are able to find out about the latest practical guidance from relevant national bodies such as the LASA (Laboratory Animal Science Association) and the RSPCA (Royal Society for the Prevention of Cruelty to Animals).

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