



Home Office

NON-TECHNICAL SUMMARY

The biology of body weight and body composition

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

Obesity, Cachexia, Body composition, Diabetes

Animal types

Life stages

Mice

adult, pregnant, juvenile, embryo, neonate

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 body weight and body composition are controlled and how disturbances in this control lead to 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?

Understanding the processes that govern body weight is highly relevant to clinical practice as disorders that arise when these processes go wrong cause a great deal of illness and suffering. Obesity, defined as excessive storage of energy as fat, is a serious issue that drives medical conditions, with obese individuals at increased risk of developing problems with their heart, blood vessels, kidney and liver, as well as being more likely to be affected by a number of different cancers. These problems bring added personal burden to the affected individual and the healthcare support needed to treat these diseases costs a lot of money. In 2017 Public Health England reported that the UK-wide NHS costs attributable to overweight and obesity would reach £9.7 billion by 2050.

At the other extreme, cachexia is a syndrome of negative energy balance where muscle and fat mass are progressively lost. It affects over a third of all cancer patients and is strongly associated with both reduced tolerance to anti-cancer therapy and reduced survival times.

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

In undertaking these studies, we will generate new data on the biological process that control body composition. These data will combine with our on-going studies in human populations and will be presented in peer-reviewed publications and shared with colleagues in academia, medical sciences and industry.

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

In the short term the major beneficiaries will be the metabolic scientific community. We also expect our research to be of benefit to the fields of cancer biology and cardiovascular disease.

In the longer term we expect our research to be of benefit to the pharmaceutical industry, where the genes and processes we identify will provide new targets for the creation of drugs to combat body weight-associated diseases with the ultimate goal that will, even in a small way, improve human health.

Finally, we believe that work under this licence will have a role in shaping and training the next generation of researchers working on understanding how "whole body physiology", the important

science of understanding how each of the multiple component parts of the body communicate to each other to allow the body as a whole to survive and thrive.

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

We will share our data and ideas with national and international collaborators and will publish our work in open access publications. We will look to share all the data we generate. We believe that it is as important to share outputs and insights that show a pathway or molecule does not have a key role just as much as it is to highlight significant insights from more novel results. We also have an active engagement with the public via lay science pieces, national newspapers and Twitter.

Species and numbers of animals expected to be used

- Mice: 17,200

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.

Human metabolic disease typically comes about because of the complex cross talk and interaction between external factors (like diet, activity and environmental conditions) and internal factors such as levels of hormones in the blood and chemical signals in the brain.

We believe that mice have the necessary, very similar organ structure and hormonal systems to humans that means studying them is going to be a really informative way to improving understanding of the "chemicals of life" like sugar and fat that are knocked out of balance in human metabolic diseases like obesity and diabetes.

Mice also offer a system in which precise changes in genes and proteins of interest can be made to really focus in on the detail of how the component parts of a mechanism all link in and work together.

As the majority of human metabolic disease affects adults, most of our work will be in adult mice. However, we recognise that these diseases only emerge after many years of exposure to particular diets and lifestyles so to be able to replicate this in a model system we will need to study younger animals.

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

The majority of the work will focus on studying animals that have been designed to either make a gene not work at all ("loss of function") or work at a higher rate of activity ("gain of function"). We particularly want to know how these changes in gene function alter the responses to environmental challenges and drugs used to treat disorders of body compositions.

These procedures can be broadly grouped into 3

- Procedures needed to create the model; the genetic engineering done in the laboratory sometimes need additional delivery of other compounds to complete the process. Whenever possible these reagents will be given by the oral route (either by mixing in the diet or by a tube inserted via the mouth into the stomach) but sometime may need to be delivered directly into the body by injection. These injections may be intravenous (into a vein), intraperitoneal (into the body cavity) or subcutaneous (under the skin). As we are focused on body weight, we will frequently change the dietary intake of an animal, both in terms of constituents and amount consumed. This can mean supplying a diet that will make the animal put on weight as well as restricting food to bring about weight loss.
- Challenges to test the system; animals will be given drugs, naturally occurring hormones and biological active reagents such as antibodies. These will mostly be delivered by injections into a vein, into a body cavity or under the skin but sometimes may require the placement under the skin of small pellet-like, devices that act as a depot for drug delivery over several weeks. Because we understand that the brain has such a crucial role in controlling body weight, we will sometime need to undertake surgery to enable us to deliver drugs and hormones directly into regions of the brain that we know control how we eat or expend energy. When we do this the mouse will be under a general anaesthetic and will be unconscious. A mouse will only usually have this kind of surgery once and the procedure takes around 20 minutes. We use a specially designed operating table that enables small sterile tubes to be placed into specific regions of the brain accurately and quickly and the mouse is usually fully recovered and back to eating and drinking within 2 hours.
- Processes needed to measure and analyse the response; animals will be placed in carefully designed study chambers and imaging scanners to measure their behaviour and response to these challenges. These special study chambers look very much like the houses the animals normally live in through the week but with some simple modifications to enable, for example, sampling and measurement of the oxygen levels in the air circulating in the chamber or measurement of how much food and water the mouse has consumed. As such, the mice can easily spend several days in these chambers without undue stress. Similarly, when the mouse needs to have a scan to check how much fat and lean tissue they have, they will be placed in specially designed tubes made of material identical to that found in the home cage to enable them to remain still and secure and quickly have a scan without the need for sedating drugs. Finally, these response measurements will sometime involve taking small amount of blood from superficial veins.

A typical experiment will involve a small series (2 or 3) of short interventions over 3-4 weeks. For example, we will give an animal a highly palatable diet for 2 weeks, treat it with a drug for a week and see what effect that had on the food intake, the body weight and the energy expenditure of the animal.

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

Most experiments proposed will lead to no more discomfort than that experienced by any rodent bred in captivity and residing in a modern animal facility.

Some animals will experience transient (seconds) discomfort when given injections or when having blood samples taken. The injections will often be of naturally occurring hormones, or compounds closely related to them. On occasion, animals may be given compounds that are recognised to produce

circulating levels of hormones that are seen in acute illness. These may reduce the animals' drive to seek out and eat food in the hours after they have been given.

When given a different diet or treatment, some animals will gain or lose weight. This will be within closely monitored parameters that take into account other important aspects of their appearance and behaviour. This weight change will typically occur slowly over weeks.

A minority of animal 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.

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)?

Breeding protocol 95% Sub-threshold, 5% mild

Other protocols

Mild- 65%

Moderate-35%

Severe- 0%

What will happen to animals at the end of this project?

- ♦ Killed

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?

Human metabolic disease is the end-result of a complex interaction between multiple external environmental factors and internal hormonal, chemical and neuronal messengers. This cannot be meaningfully replicated in anything other than animal models and although we could use non-vertebrate animals which are of lesser sentience than rodents to help in our studies, none have the necessary complexity in organ structure or wider networks to adequately address the scientific

questions posed. Further, the need for targeted genetic sophistication and the need to access deep internal tissue such as a brain requires a model organism system.

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

We look to use animal alternatives where possible. We use cell culture models for looking at specific mechanisms that occur in isolated cell populations. Results from these experiments can both be informed by our animal work and help us to design better animal experiments.

When looking to ask a question about the function of a gene found in the brain we have used neurons grown in a cell culture medium rather than use mouse brains. For example, through our links with colleagues who study human genetics, we have identified a number of possible genes that are linked to obesity. We also know from published work that these genes are expressed in the brain. These make them exciting candidates for future projects but before we do any work in mice, we want to be really sure they are working in the way we predict. To do that we have altered the function of these genes in neurons grown in a dish to see what happens, replacing the need to do these preliminary studies in animals

Further we have collaborated with colleagues who have gut “organoids” to test ideas on hormone action in the gut. Organoids are small, three-dimensional tissue cultures that can be grown in a dish in a lab into such an ordered pattern that they mimic a lot of the complexity of an organ, and remove the need to repeatedly collect animal tissue. Finally, with correct ethical approval and process in place, we have access to post-mortem material from humans to enable us to map out the location of genes of interest without using animals at all.

Why were they not suitable?

While cells and organoids grown in a dish are useful they can never generate the integrated data essential to this project. We cannot record from a nerve cell how fat it has become or if it is hungry, or determine how much an organoid has eaten. As such they not a sophisticated enough model platform to enable us to study the complex interplay between multiple organs that leads to metabolic disease.

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?

These numbers are largely based on the level of work over the previous two licenses and the amount of funding we have in place and expect to use to fulfil our aims and objectives.

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

To avoid wastage of animals, appropriate background research will be done prior to all experiments. Whenever possible, we will look to work with existing colonies of animals rather than breed new colonies. We will allow other trained researchers to work with the colonies in our unit rather than moving mice, reducing the number of mice that are both bred and transported.

Studies will be of appropriate size to detect significance, with animals randomly assigned to matched study groups and, whenever possible and practicable, investigators blinded to the nature of interventions. Protocols will include a series of analyses and steps on a single animal, rather than single analyses on multiple animals. We aim to balance impact upon an individual animal with scientific output but reason that this approach significantly reduces the number of animals used.

We think carefully about which other organs researchers in the laboratory can work on, such as heart and adipose tissue, and harvest these from the same animals. This reduces the numbers of animals used overall, increases the amount of data obtained from a single animal and allows us to examine links between different tissues/organs by combining data from the same animals, thereby enhancing the quality of the information produced.

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

We use pilot studies for any new agent we are investigating. These are used to check the safety of drugs in a small number of animals before using them in a larger cohort but also give a real world output on effect size to design the larger study.

We routinely freeze down and store either eggs or sperm from all of the different colonies of mice we study. This means we only breed animals when we need them for experiments, rather than having to maintain a permanent colony for use in potential future experiments.

We collaborate with other groups across the UK, Europe and the USA and share samples we have banked to these collaborators for their own purposes, maximising the benefit from previously conducted studies.

We are also aware that we live in an age when more and more experimental data are placed in open access platforms that are easily accessible on-line. We continue to work closely with our experts in information technology to look through these large data sets to find information that will actively shape our experimental design.

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 use mice.

We will typically use a mixture of wild-type, genetically altered and naturally occurring genetic mutant animals. Genetically altered animals are those that are carrying a specific and deliberate change in their genetic code that has been engineered to be there through laboratory manipulation of DNA.

We will use altered diets to change body weight. These are formulated to be accessible and palatable to mice.

In terms of the tests we carry out to measure circulating chemicals, either in the resting background state or after the system has been "switched on" with a challenge, we have refined protocols which deliver the smallest volume and require the smallest sample sizes possible to generate meaningful data.

Imaging and calorimetry systems are widely used apparatus that have been continually refined and redesigned over years to minimise stress. Calorimetry systems are cages plumbed in to closed circuit gas analyser systems which enable measurement of what gas a mouse had consumed and what gas a mouse has produced. In doing so, this enables a calculation to be made of how much energy that mouse has used up. While previous calorimetry systems had rather barren grid floors, continuing refinement in design and material has now made it possible to have a calorimetry system based around the existing base floor unit of the home cage.

To more accurately record food intake and metabolism, we will sometimes need to single-house mice. This can be stressful but we will minimise the time each animal is housed alone and, where possible, animals will be re-housed in groups with their original cage-mates following a period of single-housing. In addition to shelters, nest boxes and nesting material, tubes to act as hiding tunnels, shredding toys and wooden chewing toys for animals to gnaw upon will also be supplied. These will be modified as necessary to accommodate head placed cannula in any animal that has undergone surgery and had an indwelling cannula sited.

Finally, we have a number of specialised techniques that we will perform at the end of the experiment on terminally anaesthetised animals to minimise their pain suffering and distress. Animals that are under "terminal anaesthesia" remain in a state of deep sleep and unconsciousness throughout until they are humanely killed at the end of the experiment.

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

We use less sentient animals where possible and our research group has used flies to study specific research questions.

However, as our interest is in modelling human disease states, a mammalian system is the most suitable.

We do use terminally anaesthetised animals for some procedures, but under such conditions we cannot measure processes such as food intake and energy expenditure that can only be measured in live

animals over days.

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

We have made several refinements to our techniques over the past years.

Specifically;

More prolonged period of acclimatisation in an enriched environment with “same-user handling” in period prior to studies where stress is anticipated and where stress will affect data output. This has been particularly useful in studies of the stress hormone corticosterone where, without a period beforehand where the animals are used to being handled, the stress signal from the test itself may have swamped the true biological signal. Whenever possible, mice will be housed in social groups.

For calorimetry data we have taken advantage of in-house, mathematics expertise existing within our institute to factor in the inevitable change in body weight seen in the study period. This improves the quality of the data and removes the need for the animals to undergo a repeat period in the calorimetry system.

For food intake studies we have made our own dishes to capture spillage and made measurement of food intake a much faster and accurate process, maximising data yield from the time animals spend single-housed. Whenever we are using any drug that we wish the animal to take by mouth, we will work with our teams in the animal units to introduce appealing and attractive flavouring (like Strawberry Nesquik) to be delivered alongside the drug.

We will look to use close observation of both body weight and body condition scoring to get a more complete readout of an animal's situation to enable us to detect problems early and avoid harm. The body condition scoring system is a simple, rapid and noninvasive method for assessing health status and wellbeing.

In any post-operative period we will pay close attention to ensuring the environment meets the needs of the animal by the addition of a heated environment, post-operative bedding and more palatable mashed food.

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 animal research and testing (<https://www.ncbi.nlm.nih.gov/pubmed/28771074>)- used for planning our experiments

Smith *et al.* Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. *Lab Animal* 2018 Feb;52(1_suppl):5-57. doi: 10.1177/0023677217744587.

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

Our institution has a regular advisory board meeting to facilitate meaningful dialogue across the license holder, researchers and technicians. Through these meetings we are able to find out about 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).

Accessing NC3R website (<https://www.nc3rs.org.uk>) as a resource for guidelines, practical information, links to publications and training materials.

Advances in the 3Rs will be disseminated to those operating under this licence through the weekly laboratory meetings.