Home Office

NON-TECHNICAL SUMMARY

Mechanisms involved in obesity and associated metabolic diseases

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

Adipose, Thermogenesis, Obesity, Human stem cells

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

The project has an overarching theme of understanding why people became obese and then become sick (particularly why they get diabetes). Moreover we want to identify new genes/molecules that we could target with specific treatment to treat obesity and diabetes.

We have two specific objectives: The first is to determine if the negative effect of obesity can be counteracted by activating a specialised kind of fat called brown adipose tissue (BAT) Unlike the well known white fat (WAT), BAT burns fat instead of storing it. Activating BAT could be used to reverse obesity itself, or by preventing fat to go in wrong locations, diabetes. The second is to investigate how WAT function connects obesity and diabetes. Doing so we aim to identify genes/molecules that could be used as markers to predict the risk of diabetes and that could be targeted with new treatments to prevent its development.

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.

What are the potential benefits that will derive from this project?

We expect the main benefit from work carried out under this license to be in terms of scientific advancement. Our work will provide information from which other scientists and drug companies can build on to perform human studies and design new therapies. We hope to identify new genes that can be manipulated to treat obesity.

Moreover, the in vivo transplant system of human stem cell derived-cell types in mice developed in this license could be valuable to other scientists interested in organ development in human context and regenerative medicine (making new organs to replace faulty ones). Mouse fat in another mouse transplant will validate the results we obtain with the human stem cells derived-cell type in mice transplant experiments.

Findings will be made available to other scientists through publication in open access, peer-reviewed journals or on open access platforms, and presentations at scientific conferences and meetings.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use exclusively mice. We expect to use in the region of 10400 animals.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will perform experiments where mice will be transplanted with fat cells derived from human stem cells. Stem cells can turn into all cell types of the body. We have developed ways to turn stem cells into fat cells in petri dishes. In the human body, fat cells are present in different locations, such as under the skin and in the abdomen. We plan to transplant fat cells generated from the stem cells mixed with human blood vessels cells into mice and study how they will form adipose tissue. The presence of blood vessels cells will improve the formation efficiency of the fat pad. At first we will determine the best conditions for the transplant using normal cells (pilot study). We will use transplant of mouse fat into other mice to validate the results obtained with the human stem cell adipocyte transplants.

At first we will determine the best conditions for the transplant using normal cells (pilot study). Then once the methodology is set up and validated, we will investigate the impact of the transplantation of normal cells and mutated cells and the tissues that they will form on the development of obesity or diabetes in the mice. Because we will use mice having a partially compromised immune system, to perform the transplant, these mice will be maintained in a clean environment where pathogens such as bacteria and viruses are absent. According to the type of gene that we want to study, the transplanted mice could be fed diets high in fat to make them obese and/or insulin resistant (insulin resistance leads to diabetes). Some very insulin resistant models may become diabetic and drink a lot of water and produce a lot of urine. These mice will require extra care (more frequent cage changes) to prevent the development of ulcers. We perform a range of procedures that are classified as mild. These include glucose and lipid tolerance tests where mice receive a large amount of sugar or fat and we take blood samples to determine how well they can cope with it. Mice and humans with diabetes cannot deal with sugar or fat well. For some studies or procedures, such as the determination of food intake or the measurement of the energy expenditure, the mice will single housed. In most cases being alone in a cage will be well tolerated, however some mice respond to that by losing weight. To address this question the refinement measures mentioned in the refinement section will be implemented. Other procedures that we will perform are classified as moderate, such as the transplant itself and the administration of insulin to study how specific organs become insulin resistant. Very rarely animals respond badly to these protocols and may have to be killed for welfare reasons. With the transplant of cells types derived from stem cells there is a small risk of developing tumours called teratomas. That will be detected by monitoring the speed of formation of the fat pad, weight loss and levels of markers of teratomas in the blood of the mice. The mice that present sign of teratomas formation will be killed. All mice will be killed at the end of the experimental procedures.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Complex diseases such as obesity and diabetes and that we want to study and understand in this project involve the interaction and crosstalk between different organs (i.e. muscle, brain, adipose tissue, etc.). This degree of complexity cannot be recreated in a dish using current methods, and for this reason the mouse remains the best model organism to answer the metabolic questions addressed in this project.

However, we are developing a 3D culture system for the generation of organoids (mini tissues), to try to reproduce more closely the environment of the adipocytes, and even study the interaction among different cell types. Even these 3D culture systems so far cannot fully substitute for the use of animal models where the impact of mutations can be investigated at the level of the whole organism. However we are confident these 3D cultures will help us to prioritise, reduce and refine our *in vivo* experiments.

Reduction

Explain how you will assure the use of minimum numbers of animals.

The use of our human adipocyte cells model will allow us to reduce the number of mice used in our project because in principle the genes showing an effect in culture will be chosen and put forward for the animal work. Moreover, the way our project plan has been conceived will allow the use of the same animals for different aims, reducing the total number of mice to be used.

On top of that, if appropriate, both control and mutant cells may be injected into the same mice, allowing us to obtain paired comparison, increasing sensitivity and reducing the number of mice used. This is also true for the mouse fat to mouse transplant, as we will perform bilateral implantations reducing the number of animals used.

Another method to reduce animal usage will be through experimental design. By using the correct number of animals for each experiment we avoid wasting animals by obtaining either false positive or false negative results. We will determine how many animals to use based on data we already have and then use a statistical tool called a power calculation to work out the smallest number of animals we need to get a meaningful result. It is clearly important that we do not use too many animals, as then animals will have gone through the procedures unnecessarily, however using too few animals in an experiment can be even worse as we can get results which look promising, but do not provide strong enough evidence to be confident that the result we have obtained is real. In cases where too few animals are used the whole experiment has to be repeated again, wasting the animals that were used in the first experiment.

In our amendment (Oct 2020) we are adding the capacity to perform fat transplants from mouse to mouse. While in the short-term this will use more mice, in the longer term we believe this will lead to a greater reduction in mouse usage. Much more is known about how genes control fat in mice. Therefore, mouse-to-mouse transplants will let us check if the brown, beige and white fat we make from stem cells works properly. For example, if we delete a gene in our human ES cells we know affects brown, beige or white fat cell formation in mice, if our human ES cell- derived fat does not show a phenotype we currently cannot know if it was a problem with the transplantation procedure or because the gene works differently in mice and humans. Using cells or tissue from mice will allow us to check the transplant procedure is working as it should do. Equally, as mouse brown fat is more active than human brown fat, mouse-mouse transplants will act as a reference point for how much a transplanted brown fat pad can affect things like obesity and insulin resistance in our recipient mice.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Mice are a suitable species for this project because they have similar organ systems to humans which have similar metabolic functions, which are absent in more simple animals. Many mouse strains can develop the symptoms of metabolic disorders found in humans, including obesity and elevated insulin levels. The use of immunocompromised mice in this license is mandatory because of the human nature of the cells to be transplanted. The grafted mice, because of their immunocompromised nature will be handled in dedicated laminar flow cabinets and maintained in pathogen free environment. As a commitment to the 3Rs, we intend to use experimental variations which minimise the stress the mouse is subjected to. These methods are well established both in humans and in mice, and provide a way of being able to compare results between species.

Mice are social animals, so in this project they are always maintained in groups in the cage, except for specific periods and specific scientific reasons such as being able to accurately measure food intake from a single mouse. In most cases being alone in a cage will be well tolerated, however some mice respond to that by losing weight. To address this question the following refinement measures will be considered: where possible mice will be regrouped between periods of being alone. However, prolonged time as single mouse in cage could result in subsequent regrouping intolerance between males of more aggressive strains, as males begin to behave like exclusive territory owners. In this case regrouping the mice may not be possible. In less aggressive strains regrouping the mice will be attempted. Mice will be monitored for fighting. If fighting occurs to the extent that mice are injured, the aggressive mouse or mice will be removed to a separate cage. The mice are provided with environmental enrichment such as cardboard tunnels and nesting materials to facilitate normal behaviours and when they are being regrouped, some nesting material will be transferred at cage change.

Moreover, to reduce harms to the animals we employ a dedicated staff of animal technicians with specific expertise in working with mice with compromised immune system and performing metabolic analysis. They will also guarantee to the mice the required husbandry, care and welfare. The facility uses bespoke animal tracking software that help keeping good records of what is done to the mice and their health status for each project licence.

Moreover the techniques and procedures used for the metabolic analysis of the mice described in this PPL have been already optimised and successfully employed in the context of two other PPLs.