G. NON TECHNICAL SUMMARY (NTS)

Project title: Mechanisms of metabolic disease  
Duration of project - years: 5  
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES  
(b) translational or applied research with one of the following aims:  
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their  
   effects, in man, animals or plants: YES  
   (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or  
   plants: YES  
   (iii) improvement of the welfare of animals or of the production conditions for animals reared for  
   agricultural purposes: NO

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs  
   and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b):  
   NO  
(d) protection of the natural environment in the interests of the health or welfare of man or animals:  
   NO  
(e) research aimed at preserving the species of animal subjected to regulated procedures as part of  
   the programme of work: NO  
(f) higher education or training for the acquisition, maintenance or improvement of vocational skills:  
   NO  
(g) forensic inquiries: NO

Keywords:  
Obesity, Diabetes, Adipose, Fat

Describe the aims and objectives of the project (e.g. the scientific unknowns or  
scientific/clinical needs being addressed):

The project has an overarching theme of investigating why obese people become sick (particularly why  
they get diabetes). While we know obesity causes diabetes, it is not clear exactly why – simply carrying  
around 50 extra pounds all day does not make you ill, it makes you physically fit.

We have three specific objectives. The first is to determine if the negative effects of obesity can be  
counter-acted by activating a specialised type of fat called brown adipose tissue (BAT). Unlike white fat  
(WAT), BAT burns fat instead of storing it. Activating BAT could be used to reverse obesity itself, or by  
preventing fat going to the wrong locations, diabetes. The second aim is to investigate how WAT  
function connects obesity and diabetes. To do so we will investigate mouse models with increased or  
decreased WAT function in terms of a) changes in the total amount of fat that can be stored b) changes  
in when and how fast fat is stored or released. Changes in both the amount of fat that can be stored
and the speed at which it can be stored are thought to link obesity and diabetes.

We will also study how changes in the immune system link obesity to diabetes, with a particular focus on how a specific immune cell known as a macrophage becomes activated to produce harmful molecules in the obese state. The inappropriate activation of macrophages is believed to link obesity to diabetes.

Finally we will study how obesity causes liver disease. Obesity is the leading cause of liver disease (overtaking alcoholism) and it is believed that obesity causes fat to accumulate in the liver causing damage to the liver. Damage takes the form of insulin resistance, inflammation and ultimately fibrosis (known as cirrhosis) and cancer. We will study how modifying the fats stored in liver and genes that affect fibrosis control how sick livers become. Importantly, liver disease has several stages from the mildest form known as ‘non-alcoholic fatty liver’ (NAFL) through to hepatocellular carcinoma (liver cancer). Currently there is no diagnostic test to determine if a patient has the mildest form NAFL or the more dangerous non-alcoholic steatohepatitis (NASH). We will study mice and correlate our results with human studies to try to determine a marker for NAFL to NASH transition.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the 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 other scientists and drug companies can build on to perform human studies and design new therapies. Ultimately we hope to identify new genes that can be manipulated to treat obesity and diabetes. Under our liver aim we also hope to help identify a marker of NAFL-NASH transition which will help medical professionals to select patients with NASH and give them more intensive treatment.

What types and approximate numbers of animals do you expect to use and over what period of time?:
We will use exclusively mice. We expect to use in the region of 18,500 animals.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?:
Mostly we perform experiments where animals will be fed diets high in fat and/or sugar designed to make them obese and/or insulin resistant. Some very insulin resistant models may become diabetic and drink lots of water and produce lots of urine. These mice require extra care (more frequent cage changes) to prevent the development of ulcers. We perform a range of experimental 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. We also house mice are different temperatures. The lowest temperature we house mice at is 5oC. Wild-type mice can live happily at temperatures below 5oC and actually are healthier in old age when kept at this temperature. Equally, we house mice at temperatures at ‘thermoreutral’. This is a temperature where mice do not need to expend energy to make heat. Mice will select a thermoneutral environment when given a choice.
most mice thermoneutrality is ~30°C, but in some mice (such as ones with no or little fur) it can be as high as 34°C. Some mutant mice may respond badly to cold temperatures. We monitor any genetically modified mice that have not been shown to be able to cope with cold temperatures for body temperature changes using small temperature probes. Mice are removed from the cold and returned to a warm environment if their temperature falls too far. Some experimental protocols are of moderate severity. In some of these we administer either insulin or a type of carbohydrate called pyruvate 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. We also perform bone marrow transplants from one mouse to another. These experiments allow us to study a mouse with the immune system of another one. Bone marrow transplants require mice to be irradiated to destroy their own immune system. Irradiation can lead to some mice having to be killed for welfare reasons due to developing radiation sickness. Some mice will develop liver cancer, however liver cancer is largely asymptomatic and will be detected by weight loss. Mice will be killed before they develop more advanced cancer. All mice will be killed at the end of the experimental procedures.

Application of the 3Rs

Replacement:
While we try to replace mice with either in vitro models (stem cells, cell culture models) or by using lower organisms (flies), diabetes and obesity are diseases that affect humans and involve the cross talk between multiple organs (adipose tissue, liver, muscle, pancreas, brain and macrophages). As such they currently can only be studied comprehensively in mammals such as mice.

Reduction:
The main 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 have a dedicated team of support staff who are responsible for making sure each mouse has the correct genetic modification and that no unnecessary mice are bred. By making sure only the minimum required animals are bred and that they are correctly identified as wild-type or GA mice we are able to minimise wastage.

Refinement:

Mice are particularly useful models as they are readily amenable to genetic modification allowing us to study how specific genes cause disease. Like humans, mice are mammals and develop both obesity and diabetes making them suitable to study these human diseases.

To reduce harms to the animals we employ a dedicated staff of animal technicians with specific expertise in working with mice that have diabetes and obesity.

Finally, we have developed in house 'tracer' technologies. Tracers allow us to determine more information about metabolism (e.g. which organ is using glucose) without any additional suffering to the mouse under study, increasing the benefits from each mouse at no extra welfare cost.