Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 10

Projects with a primary purpose of: Basic Research – Endocrine System / Metabolism

Project Titles and keywords

1. **Regulation of metabolism & cognition: young to old**
    - VGF, small rodents, memory and learning, energy balance

2. **The Physiological Roles of Clathrin Diversity**
    - Clathrin, Biochemistry, Cell Biology, Diabetes

3. **Biological significance of endocrine cells in diabetes**
    - Glucose, Hormone, Pancreas, Gut, Diabetes

4. **The role of genes and environment in diabetes in rodents**
    - Diabetes, imaging, drugs, Mouse, Human

5. **Glucose sensing mechanisms in diabetes**
    - Hypoglycaemia diabetes central nervous system

6. **Mechanisms of adipose (fat) tissue dysfunction**
    - Adipose or fat tissue, Obesity, Lipodystrophy, Insulin resistance, Diabetes

7. **Mechanisms of metabolic regulation in health and disease**
    - cancer, metabolism, obesity, diet, imaging

8. **Mechanisms of metabolic disease**
    - Obesity, diabetes, adipose, fat

9. **Understanding energy balance in health and disease**
    - Obesity, metabolism, appetite, cachexia.

10. **Impact of exercise and high fat/sugar diet on physical and mental health**
    - Voluntary exercise, high fat/high sugar diet, epigenetics, wellbeing, prevention

Project 1	**Regulation of metabolism & cognition: young to old**	
Key Words (max. 5 words)	VGF, small rodents, memory and learning, energy balance	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Objective 1: VGF (non-acronymic) has been proposed to be involved in regulating appetite and bodyweight. Particular since mice in which this gene has been deleted from birth are lean, have increased activity and eat less suggesting that the presence of VGF would lead to obesity. However experiments in adult rodents, in which the gene is present, have suggested that this gene may be involved in preventing obesity. Thus the primary aim is to further characterise the role of this gene and its products with regards to appetite and bodyweight. Objective 2: More recently it has been suggested that VGF may be involved in the development of dementia. Indeed the levels of this gene in the brain are reduced in ageing rodents, while it's been shown to be reduced in brains of individuals who suffered with dementia. More importantly VGF has been associated with the maintenance of brain cells. Thus the secondary aim is to determine the relationship of VGF with ageing and dementia. We aim to determine whether VGF may be a potential candidate as a treatment for loss of memory and learning in both healthy ageing and in disease states.	

	Objective 3: It has been identified that obese individuals are more likely to develop dementia; in fact 40% of obese men in the UK are thought to be at risk. Additionally it has been shown that obesity decreases the volume of the brain associated with memory and learning by 8%. So the third aim is to determine the relationship between VGF, obesity and memory and learning. Thus not only has the potential to help those with dementia but also prevent decline in memory and learning in obese individuals.
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)?	Initial studies will be basic research and will lead to further understanding of how obesity and dementia is related at a gene level. However the advancement of knowledge in this area will underpin the development of lifestyle, dietary and therapeutic strategies to promote healthy aging as well as treatment for diseases such as obesity and Alzheimer's disease.
What species and approximate numbers of animals do you expect to use over what period of time?	The proposed studies will utilise rodents because they are considered to be the least neurophysiological sensitive mammals that nevertheless display fundamental mechanisms of the brain in the control of energy balance and cognition that are comparable to man. Siberian hamsters will be utilised as they have the ability to change from an obese state to a lean state by simple changing the amount of light they are exposed to. Therefore exploiting this will allow us to identify novel genes, which may be involved in regulation of energy balance. Additionally mice will be utilised as they allow genetic manipulation and many of the animal models of disease states are mice. We expect to utilise maximum 1560 animals, however before any experiment is designed, using data from similar experiments will allow us to determine exact numbers.
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?	Overall we have a moderate severity band. Thus animals may undergo surgery to help manipulate the brain or circulating system. We do not expect many adverse effects however the most prominent will be decrease in bodyweight due to poor recovery from surgery, administration of a substance at a toxic dose and possible aversion to the memory and learning behaviours. Bodyweight and food intake will always be monitored

	as well as general behaviour (changes in coat/activity/vocalisation). Weight loss is limited to a maximum of 20% of an adult mouse's free-feeding weight in age, sex and strain matched controls, and in long photoperiods to 30% of an adult Siberian hamster's free-feeding weight as natural weight loss induced by seasonal changes is of this degree. Animals are monitored and weighed at least 2 times per week. **END POINT:** Weight loss more than that stipulated then animal will be euthanized by appropriate humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are not aware of any alternative which does not use animals that would allow progress to be made towards the objective. We have considered other methods such as using human subjects and cells/tissues however these have their disadvantages. It would be very difficult for such experiments to be completed in humans; nonetheless future experiments may allow this to occur, once a suitable method of administration is determined. Effects on food intake, energy expenditure and memory and learning cannot be completed with the use of cells/tissue in a culture dish. However the experiments proposed in this project will have an element of in vitro work to complement the in vivo work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction of animal use is built into the design at several levels. • The minimum number of animals will be used; the number of animals will be determined from other similar experiments. • Experiments will be designed that were possible the animal acts as its own control. So in experiments where ageing is being investigated the same animal may be tested at a young and then old age. Where administration of substances is being investigated the same animal will receive all doses. • Where possible the most amount of information from each individual animal will be obtained. Therefore at the end of the study all tissue will be dissected to determine how genes interact.

3. 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.	Siberian hamsters will be utilised as they have the ability to change from an obese state to a lean state by simple changing the amount of light they are exposed to. Therefore exploiting this will allow us to identify novel genes, which may be involved in regulation of energy balance. Additionally mice will be utilised as they allow genetic manipulation and many of the animal models of disease states are mice. My considerable experience of conducting studies with rodents has led to effective safe procedures and regimens, in particular with respect to the type of procedures, thus minimising animal suffering, distress and long lasting harm. Any staff will be trained to be competent in general animal behaviour to be able to identify any adverse behaviour. Refinements will be brought into all aspects of design and studies will be designed so that the most of information can be obtained from each animal. • Use of automated systems: Simultaneous measurements of food intake, meal size, and length of meal, activity and energy expenditure will be used whenever possible. • Surgical techniques will be refined so that fastest recovery • Techniques for determining memory and learning will be analysed using video equipment so that the investigator can concentrate on the wellbeing of the animal. • Use of reverse lights: Rodents are nocturnal, thus to determine reductions in food intake often they are fasted as this mimics the nocturnal state. To prevent this all substances will be administered before “light off”. • Where possible substances will be administered in ways which require minimal handling, therefore use of slow releasing pellets or viruses which can induce long-term changes in gene levels.

Project 2	**The Physiological Roles of Clathrin Diversity**	
Key Words (max. 5 words)	Clathrin, Biochemistry, Cell Biology, Diabetes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Proteins are molecular machines inside cells that perform many essential functions. Like any machine, a protein must be at the right place at the right time in order to perform its function properly. We study a protein called clathrin, which is responsible for bringing other proteins from the cell's surface to the inside of the cell. Multiple molecules of clathrin assemble together, forming a coat that binds to the inside surface of the cell. The growing coat pulls the membrane to the inside of the cell, and eventually this part of the cell membrane breaks away, taking cell-surface proteins with it. The resulting sphere of membrane and protein cargo, called a clathrin-coated vesicle, can then be targeted to specific locations inside the cell. By removing proteins from the cell surface, the actions of hormones or other cellular processes can be stopped or decreased. Clathrin can thereby control the internalisation and transportation of many different types of proteins within the cell, influencing cell-cell interactions and affecting diverse physiological processes. Clathrin itself isn't just one protein, but a complex of two kinds of proteins, called clathrin heavy chains (CHCs) and clathrin light chains (CLC5). Three identical CHCs form the main units of clathrin that assemble into coat structures, while CLCs modulate	

	this assembly. In humans, there are two types of CHCs and multiple types of CLCs that combine to form different types of clathrin. In this project, we will investigate how CLC function varies in different tissues, during development, and in adult health. We hypothesize that CLCs generate differences in the properties of clathrin, thereby influencing which proteins are selected for internalisation. This allows clathrin to function in different processes in different organs. To explore this idea, we will focus on the following questions: • How does CLC variability affect cargo selectivity, and does this contribute to diversification of clathrin function in different organs? • How do the distinct functions of different CLCs contribute to development, the specialised functions of different organs, and physiological systems? We will also investigate one of the CHCs, CHC22, that we have found plays a role in the regulation of a particular protein called GLUT4, a glucose transporter important for normal control of blood sugar levels, After a meal, glucose (sugar) is transported into cells by GLUT4, in response to insulin secreted from the pancreas. GLUT4 must be at the cell surface to import glucose into the cell. When Type 2 Diabetes (T2D) develops, first cells stop importing glucose in response to insulin (called insulin resistance), and eventually the pancreas stops secreting insulin. We have found that in muscle from T2D patients, CHC22 and GLUT4 are trapped inside the cell, and GLUT4 can't make it to the surface in response to insulin. We hypothesise that this is caused by disrupted CHC22 function. We will explore this theory through the following questions: • How is CHC22 regulated — what other proteins regulate its interaction with GLUT4? • How does the molecular function of CHC22 contribute to the development of insulin resistance and/or T2D? Together, these studies will elucidate the ways in which molecular diversity of clathrin CHCs and CLCs creates Further, our functions to specialized functions of clathrin proteins. work will relate these specialized development and health.
What are the potential benefits likely to derive from	As clathrin proteins are involved in many essential biological processes, these studies have potential

this project (how science could be advanced or humans or animals could benefit from the project)?	relevance to the treatment of diverse diseases, including diabetes, cancer, hypercholesterolemia, neurological defects, developmental defects and immune function. By gaining molecular-level insight into clathrin function, we can provide important information leading to the develoomment of drugs to cure multiple diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will mostly use cells, not animals for our work. However, when we do use animals, we will use mice, as they are good models of human physiology and disease. We expect to use approximately 5,000 mice over 5 years. Many animals will be used for breeding and not in other experiments.
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?	Our protocols do not result in substantial pain, distress, or lasting harm. We will make new strains of genetically-modified mice, and while this may result in mild or moderate alterations of behaviour, body weight, or bodily functions, we do not anticipate severe defects such as seizures or death. Most of our experiments will be performed in order to identify the changes caused by genetic alterations. In some experiments animals may experience moderate anxiety or low blood sugar. Some of our protocols require that we collect organs for analysis, so mice will be given anaesthesia before being euthanised via approved methods. At the end of our studies, mice not used in further experiments will be humanely euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our initial work has indicated that we cannot recapitulate all aspects of clathrin function in cultured cells. In particular, analysis of behaviour or glucose control requires the use of animals, as these involve many organs and therefore cannot be fully modelled in cells. While we will use mice in a few of our experiments, the majority will involve cultured cells or assays where animals are only used for tissue collection and do not experience further pain, distress, or lasting harm, These are the appropriate systems for our questions, and we will only use mice when the experimental question at hand specifically requires them.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully outlined our experimental design, to ensure that we use the minimum numbers of animals while still conducting powerful experiments. At all times we will use each mouse for multiple studies to reduce the overall number of animals needed, but only if

	possible without increasing the harm to the animal.
3. 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.	We have carefully outlined our experimental design, to ensure that we use the minimum numbers of animals while still conducting powerful experiments. At all times we will use each mouse for multiple studies to reduce the overall number of animals needed, but only if possible without increasing the harm to the animal. lasting harm in the animals we use for our studies. We will use analgesia and anaesthesia where appropriate, and decide in advance humane end-points for all of our experiments, including noting unexpected phenotypes of genetically-modified mice, and stop procedures immediately if those end-points are reached. In addition, we will perform health assessments regularly and before each experiment.

Project 3	**Biological significance of endocrine cells in diabetes**	
Key Words (max. 5 words)	Glucose, Hormone, Pancreas, Gut, Diabetes	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Endocrine cells are specific type of cells that produce and release an hormone in the blood circulation to affect the function of other organs in the body. Pancreatic endocrine cells release insulin, whereas gut endocrine cells release incretins (e.g. GLP-1 and GIP). Together, these hormones play a crucial role in the digestion of dietary sugar. Diabetes is characterized by a decrease in circulating insulin and incretin levels and underlies the development of diabetes. The objective of the current project is to understand the role of endocrine cells in diabetes.	
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)?	Despite the enormous social, health and economic burden of diabetes, few new treatments are available. One reason for this is that the underlying cellular and molecular mechanisms regulating endocrine cells survival and function are poorly understood. Exploring how dysregulation of gene activity modulates endocrine cells will potentially impact on our understanding of the molecular basis of diabetes. We will also provide new fundamental information regarding organ interactions in metabolism. This may provide the basis for the development of novel therapeutic strategies aimed at fostering the survival and function of pancreatic and gastrointestinal	

	endocrine cells in diabetes. Finally, the main benefit of the work to be carried out will be the advancement of knowledge in this area through the publication of findings in peer-reviewed journals.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice: 2500/year over 5 years
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?	Our protocols will be of a mild to moderate level of severity. In brief we will be inducing diabetes with administration of diets with high caloric value and/or chemical/genetic manipulation. We will then try to reverse diabetes using drug treatment, and/or alteration of diet regiment. Treatment and monitoring of diabetes will require the use of procedures similar to those used in human patients. Many of the experiments are *ex vivo*, in which case the animal is killed to obtain tissue. In the case of *in vivo* experiments, animals are killed at the end of the experiment, typically followed by further experimental analysis (e.g., anatomical or molecular). Animals will be killed by Schedule 1 at the end of the project period. Animals exhibiting any unexpected harmful phenotypes will be killed, or in the case of individual animals of particular scientific interest, advice will be sought from NACWO, NVS or the local Home Office Inspector. If the animal fails to respond to treatment or its condition deteriorates, it will be humanely killed by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The maintenance of normal blood sugar requires the interplay between hormonal secretion from the pancreas and gut and hormonal action on peripheral tissues such as the liver, the skeletal muscle and adipose tissues. Neuronal outputs from the brain in response to changes in hormonal signalling and nutrient availability also modify the net effect on blood sugar. Such complex interrelations cannot be reproduced *in vitro* and require a whole living organism. Endocrine cells of pancreas in mouse and humans, but not lower organisms such as flies, display a unique and defined organization known as islets of Langerhans in which insulin-releasing cells are mantled by different cellular subtypes that secrete

	specific hormonal products. Islet clusters are strictly required for the function of insulin-secreting cells and cannot easily be reproduced *in vitro* using cell co-culture models or islet cell dispersion assays. Embryonic stem (ES) cell technologies reveal great potential for insulin-producing cell replacement therapies. However, more work is required to improve the maturity of those cells as illustrated by their relatively low ability to secrete insulin in response to glucose. Although we do most of our work on cell lines and freshly isolated islets of Langerhans from animals humanely killed by Schedule 1 methods, we ultimately need to validate the effects on hormonal secretion and glucose homeostasis in the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Several of the protocols that we use are designed in such a way as to obtain the maximum possible data from a single animal. For breeding genetically modified mice, where possible, we use strategies that maximise the use of offspring. When appropriate, we will cryopreserve mouse lines that are not required for extended periods, rather than maintaining stocks**.** For most of the quantitative experiments, sample sizes will be set using careful statistical analysis. We will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from other published reports). Usually 6-10 animals per treatment group are sufficient to obtain the required results.
3. 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 the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. In addition, the availability of transgenic mice provides powerful tools for examining these scientific questions. Rats give a better yield of blood and tissues per animal than mice and are preferred when we do not need to use transgenic animals. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals. Where appropriate, pain relief will be provided.

Project 4	**The role of genes and environment in diabetes in rodents**
Key Words	Diabetes, imaging, drugs, Mouse, Human
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Our current research programme has two overarching themes: 1) determine the interplay between genetics and environment; and 2) understand how this leads to diabetes.

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)?

Type 2 diabetes currently affects ~10% of the adult UK population and is associated with a significant healthcare burden. The chronically increased blood glucose levels can lead to renal and heart disease, blindness, cancer and amputation. Benefits stemming from our studies will include: 1) the identification of novel avenues for the treatment of type 2 diabetes; and 2) the production of drugs that allow better control over blood glucose levels and which display reduced side effects. The overall outcome will be the improved treatment of diabetes, leading to healthier ageing, decrease diabetes complications and reduced costs to the NHS

What types and approximate numbers of animals do you expect to use and over what period of time?

Mouse (15000) and rat (3000) over 5 years.

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?

All the protocols used in the present project are of mild to moderate severity and involve: 1) breeding of animals with specific genetic modifications associated with diabetes; 2) feeding of a high fat ‘cafeteria’ style diet to induce obesity and diabetes’;

3) clinical tests similar to those used in humans for the diagnosis of diabetes; and 4) removal of tissue under terminal anaesthesia for in vitro work. Expected adverse effects include mild discomfort from injection, anaesthetic complications, surgical complications, low blood glucose and fitting, weight loss and excessive urination. These will be recognised by good monitoring, daily checking and weighing. Adverse effects will be limited using the end points listed, e.g. failure to respond to treatment, failure to respond to glucose and weight loss (20% versus reference weight). In line with good practice, LASA or NC3Rs guidelines will be used for dosing (i.e. injection volume and needle size) and sampling procedures (i.e. blood and tissue sampling). At the end of the experiments, animals will be humanely killed

Application of the 3Rs

Replacement

The maintenance of normal blood glucose levels involves interactions between many organs (e.g. pancreas, liver, muscle and brain). Although the majority of experiments will be performed in vitro in tissue isolated postmortem, animals are sometimes required to model the complexity of glucose homeostasis, as well as determine what goes wrong during diabetes.

Reduction

The minimum number of animals required to give a valid result has been calculated using careful statistical analysis. Moreover, animal use will be minimised by validating the majority of experiments firstly in vitro, or using computer simulations. Moreover, we develop new imaging approaches which allow measures to be gathered using fewer animals.

Refinement

Mice are amenable to genetic manipulation, are easy to house and handle, and are specifically bred to be unstressed in a lab environment. Rats are sometimes used when larger blood samples are required (e.g. for measurement of hormones). Lower vertebrates such as fish and invertebrates such as flies are not relevant here, since they regulate glucose differently to humans. All the procedures in this licence are classified as either mild or moderate and are done under local, general or terminal anaesthesia, where appropriate, to minimise stress and suffering of the animals.

Project 5	**Glucose mechanisms sensing in diabetes**	
Key Words (max. 5 words)	Hypoglycaemia diabetes central nervous system	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Low blood sugar, or hypoglycaemia is a frequent complication of insulin therapy in type 1 and advanced type 2 diabetes. Patients that experience frequent hypoglycaemia eventually become less aware of developing hypoglycaemia, which leads to a much greater risk of developing severe hypoglycaemia, defined as requiring assistance from another person. Severe hypoglycaemia (SH) can cause brain damage and even death and at present, there are no therapies specifically designed to improve the awareness of hypoglycaemia. Moreover, the rates of hypoglycaemia have not changed in over 20 years, despite better treatment and insulin regimens for people with diabetes. This is because the basic mechanisms of how the brain detects hypoglycaemia are still largely unknown and require further delineation before a drug target can emerge.	
What are the potential benefits likely to derive from this project (how science could be	We expect advancement in knowledge pertaining to how the brain senses changes in blood sugar levels. Moreover, our studies are designed to ask specific	

advanced or humans or animals could benefit from the project)?	questions about potential drug targets to rule in or out potential therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats will be used up to a maximum of 1000 over the duration of the whole project.
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 expect similar adverse effects to those seen in humans that develop diabetes such as poor prevention of low blood sugars. Some adverse effects may relate to surgical procedures when taking blood from blood vessels, for example stitching may loosen causing bleeding or the tubing to remove blood may become blocked. The majority of procedures will be treatable with pain killers, antibiotics and insulin injections. The severity limit we expect our studies to go to is moderate and at the end of the study, the animals will be humanely killed and tissues will be collected to maximise the amount of information we can gain from the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do routinely use non-animal alternatives and in fact the majority of our studies do not involve animals. We have performed these petri dish studies as much as possible and have now reached the limit of what information we can glean before having to progress to more complex scenarios. In order to obtain information that is relevant to human health, we need to observe the whole body response to changes in glucose. This cannot be accurately replicated with cells in petri dishes and does not yield information completely relevant to human disease. Fortunately however, hypoglycaemia generates a similar response across many different organisms and rats provide the most widely studied species for this purpose. This means that no model validation is required.
2. Reduction Explain how you will assure	We will continue to use cell culture systems to refine hypotheses before taking studies into animal models. This means that we can be as sure as possible that

the use of minimum numbers of animals	the molecule we are interested in is likely to influence hypoglycaemia detection. Moreover, for each new experiment, we will undertake pilot studies to carry out statistical power analysis that would be required to achieve statistical significance.
3. 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.	The Sprague-Dawley rat is the most widely used model to mimic hypoglycaemia and generates changes in hormone levels similar to that seen in human diabetes. When using this model, in combination with hyperinsulincaemic-hypoglycaemic clamp studies, where we can accurately control glucose levels, we will need far fewer studies. Welfare costs are kept to a minimum by using highly trained staff to conduct all our studies. We also take advice from the local veterinary team and the animal technologists with the facility.

Project 6	**Mechanisms of adipose (fat) tissue dysfunction**	
Key Words (max. 5 words)	Adipose or fat tissue, Obesity, Lipodystrophy, Insulin resistance, Diabetes	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Obesity has become extremely common and is a massive health challenge primarily because of its links to metabolic diseases such as diabetes, fatty liver disease and heart disease. Current understanding of exactly what goes wrong in fat tissue in obese people and why this leads to the development of diseases such as diabetes, fatty liver disease and heart disease is remarkably limited despite massive worldwide research efforts. This lack of understanding is one of the reasons why so few treatment options are currently available to prevent obese or overweight people from developing diabetes and related diseases. Lipodystrophies are very rare diseases, in which fat tissue does not develop and/or function normally. They thus represent a severe example of fat tissue dysfunction and provide a unique opportunity to advance understanding of the consequences of fat dysfunction.	

	The overall aim of this proposal is to advance understanding of the pathways involved in regulating fat cell function/dysfunction as well as the health consequences of overloading or failure of this process, particularly diabetes. The approach we have adopted is distinguished by being initially based on human genetic studies involving people with lipodystrophy or obesity. Paradoxically, lipodystrophy leads to severe forms of the same diseases typically associated with obesity. In both cases, we think that these diseases arise as a result of a mismatch between the need and capacity to store surplus energy/calories in fat cells. This mismatch is particularly severe in lipodystrophy, which often makes it easier to understand exactly what is going wrong. Having made some important human genetic discoveries recently including initial evidence that mild forms of lipodystrophy are a common cause of human diabetes, we are now seeking to better understand exactly how the genetic changes we have discovered, and know are linked to altered fat function, alter metabolism and predispose to diseases such as diabetes. Recent technological developments in genetic sequencing have greatly enhanced our ability to identify completely novel genetic variants but in many cases, we know very little but how these genes affect fat tissue function and lipid/carbohydrate metabolism. Mouse models in which we alter the expression or function of these genes provide a unique opportunity to advance understanding of the role of these genes, which we know are relevant to human fat cell function.
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)?	More detailed understanding of the causes of diseases associated with fat tissue dysfunction (obesity, lipodystrophy) and their links to diabetes and heart disease. This understanding is crucial in guiding the development of useful treatment targets for these diseases.

What species and approximate numbers of animals do you expect to use over what period of time?	We will only be using mice and anticipate the need to use up to 2700 mice over 5 years.
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?	Most experiments proposed will lead to no discomfort beyond that experienced by any rodent bred in captivity and residing in a modern animal facility. The most demanding experiments involving nutrient or hormone (e.g. insulin) administration and food deprivation will lead to no more than MODERATE transient discomfort to the animals and these will affect less than ¼ of the total animals we expect to use. The animals will be humanely killed at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	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, in metabolism.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations will be made to ensure that what is deemed to be a significant effect can be detected with the number of animals assigned to that experiment. This quantitative assessment ensures that the minimal numbers of animals are used. The most important aspect of reduction is a strategic design feature: We will only progress to test in rodents ideas that are soundly supported by robust human genetic evidence. The experiments in rodents too will be structured as a 'funnel' with shorter and simpler experiments at its top; testing

	(and rejecting) ideas/hypothesis with very few ideas/hypothesis percolating to the bottom, where longer and more complex experiments will test only those hypotheses that have passed the earlier stringent rejection criteria. Thus the burden to experimental animals is matched to the potential benefit to humans and animals.
3. 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.	Subcutaneous adipose tissue is only present in higher order vertebrates, from birds upwards, so one can only really understand the biological importance of genes implicated in fat tissue metabolism in vertebrates. Of these, mice are already well established as useful model organisms for studying fat tissue development and insulin resistance. Protocols for studying these parameters are not available in birds and their metabolic homeostasis is significantly less well understood. We will only be using well-established procedures in all our studies and these will be conducted by experienced technicians. We have optimised the use of non-invasive body composition techniques such as nuclear magnetic resonance to minimise animal distress when making these measurements. The health of all animals are monitored regularly as per standard operational procedures at the university.

Project 7	Mechanisms of metabolic regulation in health and disease
Key Words	cancer, metabolism, obesity, diet, imaging
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;

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

Little is known about the ways various organs in the animal body communicate with each other using nutrients. However, there is increasing evidence suggesting that metabolic communication between cells and tissues is important for healthy tissue functions and is perturbed in disease. The aim of this project is to elucidate the mechanisms that allow cells exchange nutrients in order to support each other's functions and thereby tissue homeostasis. The project will also investigate how these operations fail or contribute to diseases such as metabolic syndrome and cancer.

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)?

The benefits of this project will be fourfold. Firstly, it will elucidate fundamental mechanisms of non-cell autonomous metabolic communication; secondly, it will reveal metabolic pathways that can be targeted for therapeutic intervention in human disease; thirdly, it will validate the use of specific compounds as therapeutic or diagnostic modalities in both non-human and human disease; and fourthly, it will generate and validate new mouse models for human disease.

What types and approximate numbers of animals do you expect to use and over what period of time?

Up to approximately 4500 mice per year will be bred and used under the auspices of this project.

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?

Experimental procedures proposed for this project have either been established or will be refined to minimise the possibility of adverse effects. Possible adverse effects expected may include weight loss, appetite loss, hunching, or temporary shivering. None of the procedures, on their own or in combination are expected to breach the moderate severity threshold. In case of unexpected adverse effects an animal care and welfare officer and a veterinary surgeon will be consulted. Any animal showing more than a moderate level of harm will be killed by an approved method.

Application of the 3Rs

Replacement

In order to generate data that is relevant to the way in which cells and organs interact with one another inside the body, it is necessary to utilise animals. For example the complex ways in which tumour cells interact with a multitude of different types of healthy host cells *in vivo* is key to understanding cancer progression but this can only be studied in a living animal. However, the knowledge acquired through this project will be used to inform suitable in vitro experiments that will aid replacement in the future.

Reduction

We will carefully plan our experiments so that to attain the best statistical power with the minimum number of animals. We will also aim to maximise the amount of information that can be acquired per animal within the confines of this licence. We will also develop or validate new, non-terminal methods that will allow longitudinal monitoring of biological parameters (e.g. liver function) in a non-invasive manner, such as *in vivo* imaging.

Refinement

Mice have been selected because of their advanced genetics, readily available disease models and well established laboratory procedures. In all cases, animal suffering will be minimised by following strict guidelines in accordance with the Home Office and by regularly monitoring animals in consultation with an animal care and welfare officer and a veterinary surgeon. Any animal showing unexpected adverse effects of any procedure will be killed immediately by an approved method. Animal use will be minimized wherever possible by employing the lowest numbers necessary to achieve statistically significant results.

Project 8	Mechanisms of metabolic disease
Key Words	Obesity, Diabetes, Adipose, Fat
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(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;

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 organ called brown adipose tissue (BAT). BAT burns lipid, instead of storing it like white adipose tissue, and could be used to reverse obesity itself, or by preventing nutrients going to the wrong locations, diabetes. The second aim is to investigate how white adipose tissue function connects obesity and diabetes. To do so we will investigate mouse models with increased or decreased white adipose 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.

Finally we will 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.

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.

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 17,500 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 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. 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. 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.

Project 9	Understanding energy balance in health and disease
Key Words	Obesity, metabolism, appetite, cachexia
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(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;

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

Health and wellbeing is dependent upon an optimal amount of body fat and muscle. Obesity, defined as an excessive storage of energy as fat, causes significant medical and socioeconomic problems. At the other end of the energy balance spectrum, cachexia, defined as unregulated breakdown of muscle and fat, is a clinical problem that reduces survival in patients with malignant and inflammatory disease.

Both conditions have unmet clinical need with meaningful intervention requiring an understanding of the processes involved.

This project aims to investigate how these disorders of energy balance can result from disruption of the critical pathways functioning in us all that control how we eat, how we metabolise fuel and how we store excess energy in our tissues.

The basis of these studies come primarily from studies of human disease, including preliminary findings from rare genetic forms of obesity, data from clinical intervention studies and also larger population based genetic studies.

We intend to extend these findings and use animal models to help gain a more mechanistic understanding of how the pathways that have been highlighted can go wrong and result in metabolic problems

We will also examine how these pathways function in the face of different external stressors such as different ambient temperature and different diets because we know that conditions such as obesity are the result of complex interplay between genes and environment.

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)?

In undertaking these studies, we will generate new scientific knowledge around the role of brain-centred pathways that control body composition. We will gain insights into the as-yet-undetermined causes of a severe wasting syndrome which, to date, acts as a barrier to successful therapy in cancer. We will also study the effects of a number of drugs that are being used in metabolic disease to better understand their site of action. These studies will benefit future interventions, being able to signpost potential strategies for therapeutic regimens with less side effects. Finally, we anticipate that we will expand our understanding of the evolving scientific field of the role of so-called “imprinted genes” and their impact on metabolic disease.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use mice. We anticipate the need to use up to 10,000 mice over 5 years

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?

Most experiments proposed will lead to no discomfort beyond that experienced by any rodent bred in captivity and residing in a modern animal facility. Some animals will experience moderate but transient 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 and are needed as a way to stimulate and study the workings of crucial metabolic pathways as giving important information on potential therapeutic uses. A minority of animal will also undergo surgery. This will involve placing small cannula into specific regions of the brain and placing bespoke drug delivery devices under the skin and may undergo moderate, limited discomfort in the immediate perioperative period. However, this will be minimised by administration of pain killers. All animals will be humanely killed at the end of the experiments and tissues taken for further analysis.

Application of the 3Rs

Replacement

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 increasingly use non-vertebrate animals like flies and

worms which are of lesser sentience than rodents to help in our study, none have the necessary complexity in organ structure or wider networks to adequately address the scientific questions posed.

However, we continue to replace animals whenever possible and have successfully done so using yeast assay systems and neuronal cell culture lines as alternative methods to animal models. We have also begun studies in fruit fly models and developed a screening method to further study genes relevant to metabolic disease that have been identified in human population genetic studies. These will provide invaluable data to provide focus in future work and replace the need to undertake such screening in mouse models.

Reduction

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 allow other trained researchers to work with the colonies in our unit rather than moving mice, reducing the number of mice that are bred and transported. Studies will be appropriately statistically powered prior to commencing. Protocols will be planned with a series of analyses and steps undertaken on a single animal. We aim to balance impact upon an individual animal with scientific output but reason that this approach significantly reduces the number of animals used.

Refinement

Rodent models allow access to metabolically relevant tissues, like the brain and pancreas, that remain, due to ethical and practical considerations, inaccessible in human studies. Rodents have well defined pathways that both match those in humans and are readily amenable to genetic manipulation. To minimise impact on welfare we will use enriched, size appropriate housing; we will use refined standard methodologies in experiments; we will minimise pain using non-invasive techniques whenever possible and using pain relief as required; we have embedded in study plans both steps for monitoring and early detection of potential side effects and should they be encountered, to enable us to apply early humane end points.

To enable accurate measurement of food intake, sometime mice may be singly housed. During this period, there will be appropriate steps to enrich the environment. Shelters, nest boxes and nesting material will be supplied as standard. Tubes to act as hiding tunnels and shredding toys and wooden chewing toys for animals to gnaw on will also be supplied. When not having food intake actively measured, food will also be hidden in bedding and floor covering to give the animals the opportunity to forage.

Project 10	**Impact of exercise and high fat/sugar diet on physical and mental health**	
Key Words (max. 5 words)	Voluntary exercise, high fat/high sugar diet, epigenetics, wellbeing, prevention	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand at the molecular and cellular level how voluntary exercise and/or high fat/sugar diet and! affect physical and mental health at different ages. Using a rodent model of voluntary exercise we will study: A) the epigenetic impact of voluntary exercise in the brain; B) how these epigenetic changes correlate with changes in gene expression, stem cell function and behaviour; C) whether the epigenetic and stem cell effects of exercise in brain are different than in other tissues (i.e. adipose tissue or muscle); D) how exercise can prevent the negative epigenetic effects of high fat/sugar diet in brain and body, and how does it translate at the stem cell (neurogenesis & adipogenesis) and physiological level (metabolic syndrome); and E) identify blood markers of brain function (underlying the effects of exercise and/or	

	high fat/sugar diet).
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)?	This proposal will widen the understanding of how exercise and/or high fat/sugar diet affect the changes in epigenetic markers and adult stem cells in brain, muscle and adipose tissue. It will also provide us with a list of possible blood biomarkers of the effects of exercise and/or diet on brain function (therefore potentially decreasing the need of future animal experiments). These findings will serve as a springboard for future studies aiming to develop therapies boosting or mimicking the positive effects of exercise on healthy ageing and mental and physical health.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice totalling 550
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?	All our procedures are within the moderate severity and, based on previous experience, adverse effects are unlikely. The behavioural tests that we will perform are mild or moderate. Based on previous experience in our laboratory and across the woild we do not expect adverse effects from giving the mice voluntary access a running wheel (on the contrary, access to the wheel will enable the animal to reach the levels of physical activity it should have had in the wild, which are prevented by being housed in a cage). Rodents are routinely maintained on high fat/sugar or “cafeteria” diets as a model for metabolic syndrome, which is highly prevalent in human population. Therefore we anticipate the mice will develop obesity, mild hypertension and insulin resistance, but as relatively short term studies are planned this should not majorly impact the animal’s welfare. At the end, the animals will be euthanized humanely

	and their brains, organs and body fluids will be studied ex-vivo.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of living animals is essential as the overall objective of the research is to gain greater understanding of the impact of voluntary exercise on brain, body and behaviour. It is also not possible to use human based studies, as it is not ethically feasible to obtain biopsies of tissues such as brain. We will use mice, as many cellular, molecular, physiological and behavioural mechanisms are similar between mice and humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Making use of the available literature and pilot data we will perform to perform power analysis in order to keep the number of animals used to a minimum. An advantage of our experimental design is that often we will study the effects of exercise and/or high fat/sugar diet on several tissues from a single animal. Thus requiring only one group of animals, instead of several.
3. 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.	Replicating the effects of exercise across the body requires a mammal, and the mouse is the lowest sentient species suitable for this purpose. We will use well established protocols to produce minimum discomfort to the animals. The welfare of each mouse will be monitored routinely. In the unlikely case of adverse effects linked to any of the experimental procedures will be controlled by expert personnel that will monitor the animals carefully.