Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 6

Projects with a primary purpose of: Translational and Applied research – Human Endocrine or Metabolism Disorders

Project Titles and Keywords

1. **Treatment of metabolic disorders**
    - Obesity, Diabetes, Nephropathy, Neuropathy, Retinopathy

2. **Optimising Islet Transplantation in Type I diabetes**
    - Diabetes, islets, beta cells

3. **Biomarkers of Drug Dependence and Behavioural Endophenotypes**
    - Addiction, neurobiology, neuroimaging, vulnerability

4. **Molecular mechanisms of CFTR channel gating**
    - CFTR, ion channel, protein conformation

5. **The role of the microbiome in nutrition and metabolic diseases**
    - Bacteria, nutrients, obesity, diabetes, liver

<table>
<tr><td>PROJECT 1</td><td>Treatment of metabolic disorders</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Obesity, Diabetes, Nephropathy, Neuropathy, Retinopathy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Drugs currently available to treat metabolic disorders are limited by poor efficacy and/or unacceptable side-effects. More efficacious and safer drugs to treat metabolic disorders are urgently required. The main purpose of this project license is provide highly specialised preclinical services to the pharmaceutical and biotech industry to evaluate the efficacy, mode of action and side-effects of novel drugs and novel pharmacological targets for the treatment of metabolic disorders. There is a real demand for these services from pharmaceutical and biotech companies due lack of appropriate expertise or laboratory facilities in-house and/or capacity issues.</td></tr>
<tr><td>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)?</td><td>It is anticipated that experiments conducted under this licence will expedite the development of improved drugs to treat metabolic disorders, allowing faster access to safe and efficacious drug therapies for serious medical conditions such as diabetes and its complications. Wherever possible, information will be disseminated into the scientific community. This will further knowledge about novel molecular targets in the CNS and periphery and the efficacy and/or safety of new drugs to treat metabolic disorders.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Approximately 8000 rats and 8000 mice over 5 years</td></tr>
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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?	The majority of studies to investigate the efficacy, mode of action or side-effect profile of drugs will involve simple dosing (single or repeated) by an appropriate route with blood or tissue sampling for pharmacokinetic, biochemical, histological or neurochemical analysis and/or behavioural/physiological testing. Some drugs may already have been tested in vivo by the client (before being sent to us for evaluation in models that the clients do not have themselves) and would not be expected to produce any adverse effects. Occasionally, substances will be evaluated which may not have been tested in animals before and may produce unexpected toxic effects which could cause pain, suffering and lasting harm or in extreme cases death if humane end-points were not applied. Occasionally, drugs will be given centrally or peripherally by continuous infusion from osmotic minipumps or administered directly into the brain or a vein. These methods will involve anaesthesia and/or surgical procedures for subcutaneous implantation of minipumps, indwelling cannulae into the brain or intravenous catheters and it is possible that post-surgical complications may occasionally arise or the animals may experience post-operative pain. In some cases, the animal models employed may involve induction of diabetes, or obesity and/or involve training in specialised equipment which may produce transient discomfort/stress. For these reasons, the likely/expected level of severity of the license is moderate. At the end of procedures animals will be terminated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are no alternatives to the models employed as they are used to assess the integrated behavioural and/or physiological/pharmacological responses of the whole animal to different treatments. All drugs to be evaluated will have been extensively characterised in vitro. However, as information obtained in cell-lines, cells and tissues relates to only part of the animal, it cannot replace ex vivo or in vivo tests. Such animal testing is a fundamental requirement for progressing novel agents into man and for dossier submission to the regulatory authorities.
2. Reduction Explain how you will assure the use of minimum numbers	Animal numbers will be minimised by only testing drugs in assays relevant to their pharmacological profile; measuring several parameters in the same

of animals	animals wherever possible and continued use of animals (where animal welfare and experimental data will not be compromised). A fully qualified, highly experienced biostatistician will advise on experimental design and ensure that the minimum numbers of animals are used to produce meaningful statistical comparisons.
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.	Rats and mice will be used as details about their endocrine and central nervous system have been well-documented and they are the lowest form of mammal that can provide meaningful data about man. Typical studies will employ normal healthy animals (generally adult, on rare occasions as young as one month). Occasionally, genetically-altered animals may be used to model specific metabolic disorders or provide proof of concept for novel targets for treatment of metabolic disorders. Animals displaying adverse phenotypes will not be used. A variety of established, fully-validated animal models and assays will be employed. These have been widely used by the pharmaceutical industry to predict the effects of drugs in man. Animal models that produce unnecessary suffering will not be used. Substances will be given by the least severe route of administration. If substances have not been given to animals before, pilot studies will be performed. Simple acute screens will normally be conducted before chronic or complex assays. In general, the behavioural/physiological tests employed are well-tolerated. Exposure to these tests will be kept to the minimum necessary to obtain the data or for the animal to learn a given behaviour. If substances are given as part of the procedure to induce diabetes (e.g. the use of streptozotocin) or a specific pharmacological response, doses will be carefully chosen and experiments designed so that any adverse effects and/or the duration of time that animals are exposed to the adverse effects are the minimum required to enable scientific objectives to be met. Surgical procedures will only be used if alternatives are not available. Anaesthesia will be maintained at a suitable depth to avoid the animal feeling pain. Aseptic operating procedures, topical application of suitable antiseptics and plastic dressing will be used to reduce the possibility of infection. Post-operative analgesia will be used as advised by the NVS to reduce pain and suffering. All animals will receive the highest possible standard of post-operative care. The project is supported by a

	dedicated animal husbandry and technical support team. Studies will be conducted by staff highly-experienced in animal handling. Animals on study will be monitored closely and veterinary advice will be promptly proveded should it be needed. Consideration will always be given to ways to minimise any welfare costs to the animals.

PROJECT 2	**Optimising Islet Transplantation in Type I diabetes**	
Key Words (max. 5 words)	Diabetes, islets, beta cells	
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[3]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Diabetes is a condition characterised by high sugar levels which can lead to multiple health complications. People with Type *I* diabetes are usually dependent on insulin injections to control their sugar levels. The pancreas contains islets a group of cells that under normal conditions can secrete insulin. Islet transplantation, a technique involving placing islets from a donor pancreas into a patient with type I diabetes, is a treatment for extremely poorly controlled diabetes. However, there is a shortage of donor pancreases, secondly, more than 60% of the islets which are transplanted into the liver fail to engraft in the first three days following islet transplantation. Therefore most patients require two or more islet transplantations to achieve a beneficial clinical response. Furthermore in the longer term islet function diminishes and little is known about the effects that cause these changes. In addition as the adult population is becoming more obese	

	consequence of this is that more people are developing fat in their liver which may progress to become inflamed with a degree of fibrosis. This project's main purpose therefore is to develop techniques to optimise islet engraftment in the context of a normal, fatty and fibrosed liver. The second purpose is to evaluate the function of "manufactured" cells made into insulin secreting cells.
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 main benefits are scientific/knowledge based in the first instance leading in the longer term to clinical benefits. This project would mean that potentially only one donor pancreas would be sufficient per person. Therefore more donor pancreases would be available for more people. Furthermore, alternative strategies to achieve insulin secretion would be evaluated.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat (including BB rat) mouse including humanised mouse models and other immunodeficient (NOD SCID) mice <3700 mice over 5 years <1300 rats 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?	The potential adverse effects in this project are mainly of surgery, and medicine administration. Deaths resulting from anaesthesia or surgical complications are uncommon (<1%) and will be minimised by correct dosing of anaesthetics, by accurate weighing and by maintenance of body temperature during and post surgery e.g. use of heat pads. Pain will be controlled during surgery by general anaesthesia and post surgery by analgesics. Surgical infections are rare and the risk minimised by good surgical and aseptic techniques, Surgical sites will be monitored for signs of infection and medicines including pain relief given as appropriate. Within any experimental procedure administering/transplanting insulin producing cells, there is a risk animals may go hypoglycaemic, with the potential for fitting, seizure or death. Careful monitoring of blood glucose levels, and therapeutic administration of dextrose, should allow reversal of hypoglcycaemic events. With respect to specific models the main adverse effect with respect to: 1) the diabetes models is

	weight loss and excessive diuresis and thirst — animals will be closely monitored. All animals that have diabetes will be treated with some form of insulin and therefore we expect side-effects to be extremely low; 2) carbon tetrachloride model — this may cause drowsiness initially and the animals may appear unwell for 24-48 hours afterwards however their condition will be closely monitored during this period. Over 95% make a full recovery. At these doses we expect a mild form of fibrosis in the liver but we do not anticipate ascites however if this does develop then animals will be killed humanely. 3) Partial hepatectomy: in the post-operative period there may be an increased risk of blood loss although the group here has much expertise in this technique and therefore this is seldom seen here. 4) Methionine choline deficient diets— these diets generally cause increased fat in the liver but they may cause rapid weight loss (this may occur in <10% of cases). Weight will be monitored regularly. Animals will be humanely killed at the end of the study so that tissues can be analysed. 5) Kidney ligation/nephrectomy: removal of a kidney may cause hypertension, or expansive remodelling of the remaining kidney. During the time frame of our experiments we don't expect these to be problematic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It would not be appropriate to use humans for these experiments as this would involve multiple liver samples taken surgically removed, a technique that is associated with a high risk of bleeding. Mice that have been transplanted with islets into the liver reflect key aspects of the transplant process in humans. The use of genetically modified animals as well as interventions that are not possible in humans allows us to dissect the different contributions of hormones, nutrition and immune cells to islets engrafting into the liver. This research can provide vital data to enable treatments in humans. Our investigations in live experimental animals are supported by extensive analyses of tissues taken

	once the experiment is complete and are complemented by investigation of isolated cell systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used in our investigations is based on power calculations to determine optimum group size and statistical power. Where possible, a multi-factorial design is used to increase power and reduce the overall number of animals required. The use of inbred mice reduces experimental variability and thus overall numbers required. Imaging techniques (similar to those used in humans) in live animals allow sequential non-invasive measurements, providing repeated measures within a single animal, increasing statistical power and reducing the number of animals required for experiments. The effects of treatments are based on comparison with appropriate control and/or sham treated groups. Study design is based on current best practice and, where necessary, following discussion with statisticians.
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.	In all our experiments we are mindful of the need for refinement to reduce suffering, and appropriate modifications to protocols will be incorporated where possible. In carrying out experiments in rodents, we will always seek to incorporate these refinements. We will observe carefully for signs of stress in the animals.

PROJECT 3	**Biomarkers of Drug Dependence and Behavioural Endophenotypes**	
Key Words (max. 5 words)	Addiction, neurobiology, neuroimaging, vulnerability	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals[4]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The project aims to indentify and assess the causal relationship of biomarkers for enhanced risk for the development of drug dependence and associated behavioural endophenotypes	
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)?	Understanding how the brains of susceptible individuals are different from those who are apparently resilient to dependence may provide novel targets for treatment strategies to better treat drug addiction. Further the continued application/development of neuroimaging techniques for the study of neuropsychiatric disease states will improve the ability of these techniques to detect clinically relevant findings, and ultimately aid in translation of basic research.	

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What species and approximate numbers of animals do you expect to use over what period of time?	It is expected that 1700 rats will be used through the duration of the 5 year 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?	Adverse effects of the techniques proposed in this project are expected to be rare however there are certain risks associated with the use of anaesthetics, surgery and intra-venous drug self-administration relating to infection. A close working relationship between research and animal house staff, the NACWO and NVS working on the project and continued refinement of procedures will ensure these adverse effects are kept to a minimum. Only 60% of the animals are expected to undergo surgery and will reach moderate severity, and none are expected to exceed this. In the case where aversive conditioning stimuli are used to illicit a change in behaviour (18%), animals can avoid the aversive stimulus by learning to avoid a punished response. Animals exposed to aversive stimuli may exceed moderate severity if they fail to learn to avoid the punished response, but in our experience on previous licences this is observed in 20% of animals (3% total) and critically models the classic definition of addiction as drug use despite negative consequences. Suffering is minimised by using the fewest exposures and lowest intensity possible (while still resulting in robust behaviour for analysis). Following completion of the experimental protocol animals will be culled via schedule I or perfusion to enable the collection of relevant tissues for further scientific assessment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Rats represent an ideal species in which to conduct such an experiment as they readily self-administer drugs and exhibit behavioural traits in a manner that is homologous with humans. There are no alternative or less severe models that allow answers to these important questions to be obtained. Such questions can not be addressed in humans due to issues associated with assessing individuals prior and during the development of addiction.

2. Reduction Explain how you will assure the use of minimum numbers of animals	This programme of work utilises the non-invasive technique of neuroimaging to investigate pre-existing and drug induced alterations in the structure and function of the brain. Such an approach significantly reduces the number of animals required to conduct such an experiment as an individual can be assessed at multiple time points. This approach also offers the advantage that animals act as their own controls, further reducing numbers required. All protocols are well-established within the research facility and will be conducted by competent individuals with sufficient experience in the technique being performed, further reducing numbers due to experimental error. Appropriate statistical calculations based upon results from previous work within the laboratory have been used to ensure the minimum number of animals are used while still obtaining meaningful 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.	Rodents are the least sentient organism that can model the complex behaviours of interest. The behavioural tasks chosen have been developed in this laboratory and are widely acknowledged internationally as modelling specific aspects of drug use relevant to understanding drug addiction. Where aversive stimuli are used, suffering is minimised by using the fewest exposures and lowest intensity possible (while still resulting in robust fearful behaviour for analysis) and the majority of these animals learn to avoid the aversive stimulus by inhibiting certain behaviours. We have additionally adopted the principles of the LASA guidelines regarding animal surgery, and are continually refining our techniques. A close working relationship between research and animal house staff, the NACWO and NVS working on the project will ensure that a high standard of animal welfare is maintained and minimum severity induced, enabling the acquisition of biologically meaningful data.

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<tr><td>PROJECT 4</td><td>Molecular mechanisms of CFTR channel gating</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>CFTR, ion channel, protein conformation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
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<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td>~~Yes~~</td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Cystic fibrosis is the most common fatal inherited disease in the UK. It is caused by low or absent activity of a protein called CFTR. On the other extreme, excessive CFTR activity is responsible for both dehydration during secretory diarrhoeas (e.g.cholera) and polycystic kidney disease. CFTR is a channel: it provides a pathway, specific for anions, to cross membranes. It can assume various positions, called states, which can be either open (i.e. anions can flow) or closed.
Here we propose to study in depth how CFTR works. We will use two distinct, complementary, approaches. In Aim 1 we will focus on opening of the channel. We will use an established analysis, REFER, in a novel way. Our REFER study will investigate the temporal sequence in which different parts of the protein move during channel opening and, in particular, how a particular “joint” of the protein (the NBD/TMD interface) moves. This analysis will give information on the structure of an unstable, transient state called transition state which determines how frequently the channel opens.
In Aim 2 we will also focus on the NBD/TMD interface but we will extend our studies to steps following opening in the CFTR functional cycle. We have preliminary evidence suggesting that a movement at the NBD/TMD interface might occur while the channel is open, during a step which is followed very rapidly by channel closing (and</td></tr>
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	therefore controls how fast the channel closes). Our investigations will allow us to determine how strongly two sites on opposite sides of the NBD/TMD interface interact, and on how this interaction changes during the various steps of CFTR's functional cycle. The results of experiments in Aim 2 will therefore allow us to determine the direction, as well as the timing, of movements occurring at the NBD/TMD interface during the entire gating cycle
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)?	Over the past 10 years academic efforts (including ours) have led to a major breakthrough in understanding how CFTR works. We now also have some information on the 3D shape of CFTR and in particular on two positions it is likely to assume. But these are only snapshots, we do not know yet at what point of the functional cycle CFTR takes these positions, nor how or when the protein moves to go from one position to the other. Modulating CFTR function would be beneficial for treatment of several diseases (cystic fibrosis, secretory diarrhoeas, polycystic kidney disease). At present there is a large gap between industrial efforts to obtain compounds that affect CFTR activity (based on random high-throughput screening of compound libraries), and academic research aimed at understanding how the CFTR protein works. While screening has identified several promising compounds, unfortunately, the wealth of information that has emerged from basic research has yet to have an impact on drug discovery. Here we will address that gap: we will concentrate on one area of the protein, the NBD/TMD interface, which could be a good binding site for a CFTR-specific drug, and on two strategic steps in the functional cycle which are best suited as intervention points for altering CFTR activity (the opening step and the step triggering closure). Together with structural data, the results of our investigations could lay the foundations for the rational design of CFTR potentiators and inhibitors. In addition, many other transport proteins are closely related to CFTR (ABC transporters). We will use the ion channel CFTR as a model, to probe the conserved mechanism by which these proteins work. ABC transporters play fundamental roles in diverse physiological processes. For instance, they determine tissue distribution (preventing penetration in brain, fetus, lymphocytes and tumours) and oral bioavailability of most therapeutic

	drugs. Thus our studies might also suggest ways of beneficially altering the activity of other ABC proteins (e.g. inhibiting ABC transporters involved in keeping therapeutic HIV/AIDS drugs out of lymphocytes and brain). Thus, while the importance of the studies we propose lies mainly in a deepening of our biophysical understanding of a molecular mechanism (how CFTR and ABC proteins work) our proposed studies, in the longer term, also have the potential to improve the health of different patient cohorts (CF, secretory diarrhoea, central nervous system disorders, HIV/AIDS, cancer).
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to a maximum of 20 adult female Xenopus laevis frogs per year for 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?	The protocol should not cause any pain to the animals. The main adverse effect expected is distress due to capture and transfer to anaesthetic solution, prior to loss of consciousness. Following the removal of ovarian tissue, the frogs are euthanized, while still anaesthetized (unclassified level of severity).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our study requires a large number of different mutant versions of CFTR, i.e. of a complex protein, which works only when properly embedded in a biological membrane. Artificial systems achieving this are far more expensive and labour intensive.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Ovarian tissue from a single frog is sufficient to provide material for several labs. However, the material can be studied only for a limited time (~ 5 days). To reduce the number of frogs sacrificed several different laboratories coordinate their operations, so the ovarian tissue obtained from each operation can be shared.
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 are not aware of equivalent methods utilizing less-sentient animals. Cell lines require a much higher investment in staff time and money. To minimize suffering the frogs are deeply anaesthetized prior to removal of ovarian tissue. To eliminate the pain and suffering of healing from surgery we do not request permission to use a single animal for two successive operations.

<table>
<tr><td>PROJECT 5</td><td>The role of the microbiome in nutrition and metabolic diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>bacteria, nutrients, obesity, diabetes, liver</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The gut bacteria are known to be involved in many diseases such as obesity, diabetes, heart diseases and liver diseases. However, the underlying mechanisms are still unknown. Weight loss surgery has become increasingly popular in treating morbid obesity and diabetes and it causes gut bacterial changes, which could be the key player in reducing body weight and type 2 diabetes (T2D). The main aims of this project proposal are to establish microbial transplant models and study the interactions between the host, gut microbiota and nutrients in disease and health status.

Objectives: (1) to investigate the biochemical responses of animals to caloric restriction, high fat diet, choline-deficient diet, choline chloride or phosphatidylcholine supplement; (2) to investigate</td></tr>
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	the microbial changes after dietary interventions; (3) to investigate the biochemical (including fat distribution) and bacterial changes after the gut bacterial manipulation.
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)?	**Academic Beneficiaries** This project will greatly benefit researchers in the field of host-microbial interactions in health and diseases. The resulting microbial transplant method will extensively benefit scientists working on the microbial functionality and microbial manipulation. I will present the results regularly in departmental seminars and meetings in the Centre for Digestive and Gut Health at Imperial College, where audients are from both clinical and scientific units. The findings will further our understanding of the role of the gut microbiota in metabolic diseases and lead to discoveries of new non-surgical treatments and a non-invasive way of treating these metabolic diseases. **Economic and societal impact** Although the current project focuses on the fundamental aspect of host-microbial interactions in metabolic diseases, the research outcomes of weight loss and type 2 diabetes improvement responsible microbes will potentially promote the development of new treatment strategy. Modulating these functional microbes by diet or lifestyle could potentially contribute towards a knifeless treatment strategy for obesity and diabetes. Establishment of microbial transplant in conventional animals will provide alternative approaches to explore the exciting area of host-microbial interactions.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years Wistar rats (n=640) Zucker Diabetic and Fatty rats (n=260) Zucker Lean rats (n=260) C57BL/6J mice (n=360) C57BL/6J *Apoe*$^{-/-}$mice (n=360)
In the context of what you propose to do to the animals,	A successful weight loss surgery on fatty and diabetic rats or mice and transfer their gut bacteria

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	into a new batch of fatty and diabetic or only diabetic rats or mice to monitor their weight loss and T2D resolution, which make it possible to assess the impact of these gut bacteria on the rats without surgical intervention. Dietary interventions will be performed to evaluate the gut bacterial responses to different types of food. **Adverse effects** *Bariatric surgery:* We would expect the animals to lose weight (25%) as a consequence of the surgery. If animals experience pain, they will be given analgesics. Rare re-opening of wounds may be treated by re-closure under short-term general anaesthesia on one occasion only. Aseptic techniques will be applied prior to the surgery. Vitamins and minerals will be given to maintain animal health. *Metabolism cages:* Initially animals might be agitated (e.g. decreased water and food intake, making excessive noise) when placed in the metabolism cages. However, most animals quickly become accustomed. Animals that do not will not be used on that occasion. *Oral gavage of antibiotics and saline solution:* We do not anticipate any adverse effects. In the rare cases of lung dosing or oesophageal ruptures, animals will be culled by Schedule 1 methods. *Oral gavage of* caecal/faecal slurry or bacterial cultures*:* we would expect the animals to lose weight as a consequence of the transplanted microbiota. But the body weight loss would be expected around 15% with a maximum of 20%. If animals experience pain (extremely unlikely) and body weight loss (20%), a Schedule 1 method will be applied. *Dietary intervention:* we do not anticipate any adverse effects. However, if animals experience weight loss 20% or pain, they will be excluded from the intervention and schedule 1 method will be

	applied. *Imaging:* The animals' physiological status will be continually monitored throughout the experiments using appropriate monitoring equipment, and the experiments will be immediately terminated if signs of anaesthetic complications/ physiological distress are observed in the animals. **Humane end points** All animals will be killed by a Schedule 1 method if they experience excessive weight loss (30% in protocol 1; 20% in protocol 2), after completion of the experiments or animals reach humane end points as defined in the score sheet.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The main emphasis of the project is on the interactions among diet, microbiota and the host, which cannot be achieved without using animals. We have been doing studies in obese patients who underwent weight loss surgery and generated some hypotheses of postoperative weight loss and bacterial establishment, but it is not ethical to conduct further experiments on humans (e.g. transplanting faecal bacteria from bariatric surgical patients into an obese patient) to validate these hypotheses. Although faecal transplant for treating *Clostridium difficile*-infected patients is clinically used, such microbial treatment methods are yet to be applied for other disease treatment. To further develop this area and move it towards clinical application, the animal experiments are crucial at this stage. Therefore, there is no feasible alternative that would replace the animal use.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have consulted the departmental statisticians on experimental design and group size in order to minimize the number of animals used and maximise the information gained. Standard operation protocols will be established for all experiments and procedures and researchers will be ensured to fully understand the experiment objectives, experimental procedures and potential

	health and safety risks.
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.	Weight loss surgery, the gut bacterial transplantation via oral gavage, dietary interventions and body imaging will be performed on animals. To minimise animal suffering, antibiotics may be used pre- and post-surgery to prevent animals from surgical complications. Anaesthetics will be used to minimize the pain and liquid food will be fed postoperatively to ensure animal health. Sham-operated animals are expected to experience less pain and discomfort but the same treatment will be applied.