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NON-TECHNICAL SUMMARY

Molecular mechanisms in cardiometabolic disease: effects of diabetes on blood vessels

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Cardiovascular disease, Diabetes, Obesity, Angiogenesis, Aneurysm

Animal types	Life stages
Mice	juvenile, adult, neonate, embryo, aged, pregnant

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

This project aims to identify the molecular mechanisms which lead to the development of diabetes and cardiovascular disease. It focuses specifically on how diabetes affects the risk of developing diseases of blood vessels and the circulation including atherosclerosis, narrowing of blood vessels after treatment, and aneurysm formation.

A retrospective assessment of these aims will be due by 26 October 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cardiovascular disease is the commonest cause of death in people with diabetes. Although much is known about some of the links between diabetes and diseases of the heart and circulation, we still do not fully understand why people with diabetes remain at high risk of cardiovascular disease and do not respond as well to treatment. Increasing our knowledge in this area is vitally important at this time, because changes in human lifestyle have led to large numbers of people with obesity and are predicted to cause a huge increase in the number of people worldwide with diabetes over the next 15 years.

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

We expect to have increased our understanding of the causes of blood vessel disease and how these are affected by diabetes. In particular we will better understand the actions of insulin and related proteins within blood vessels and how these are altered by both diabetes and cardiovascular disease. We hope to have identified new genes or proteins which link diabetes with cardiovascular disease.

Our short time outputs will be scientific papers published in scientific journals and presentations to the scientific community at meetings. We hope that our research findings will allow us generate longer term

outputs with new ways to diagnose, prevent and treat cardiovascular disease in people with or at risk of diabetes.

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

In the short term the scientific community will benefit from these outputs, which will increase our understanding of the basis of cardiometabolic disease. In the longer term we hope that our outputs will improve the lives of people living with, or at risk of, diabetes and cardiovascular disease.

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

We will maximise the value of our outputs by dissemination through a variety of means. These include presentations at scientific meetings, publications in open-access scientific journals and release of key findings through our institution's websites and social media streams. We have close links with networks of researchers and clinicians working in this field. Our institution has strong support systems in place to facilitate translation of research findings through to clinical application. We work very closely with colleagues in other disciplines - for example to allow us to develop new drug-like molecules to explore the findings from this research.

Species and numbers of animals expected to be used

- Mice: 10 600

Predicted harms

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

Explain why you are using these types of animals and your choice of life stages.

We use mice to study the links between diabetes and cardiovascular disease. This is because it is relatively straightforward to alter the genes of mice and study how that gene influences diabetes or affects blood vessels. Many lines of mice are available to the scientific community in which selected genes have either been deleted or increased. Because genes encode proteins in the body, this allows us to study the effect of specific proteins in diseases. One example is that we study the receptor by which insulin exerts its effects on the cells of the body. By reducing or increasing the numbers of insulin receptors in certain cells within the blood vessel wall, we can see how insulin and its effects on those cells influence the susceptibility to blood vessel disease.

Mice are amenable to studying the effects of most of the diseases that affect humans. Because mice are mammals, findings can be used to mimic what happens in humans. For example, we can study diseases such as atherosclerosis (furring up of arteries), peripheral arterial disease and aneurysms as well as reproducing the effects of treatments such angioplasty. We can examine how the influence of diabetes on blood vessels affects the body's capacity to repair and heal itself, for example in healing of

wounds and the growth of new blood vessels. Finally, we can learn how diabetes in pregnancy affects the susceptibility to diabetes and cardiovascular disease in children.

The majority of our research is carried out in adult mice. We use neonatal mice to study the development of blood vessels in the retina. We also study the effects of genes in pregnant mice on the placenta and the developing fetus.

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

Mice used in this project will typically have been bred in another project licence held by the applicants to create genetically altered animals (Molecular mechanisms in cardiometabolic disease: breeding and maintenance of genetically altered animals: PP5104353). The genetic alterations affect the animal's molecular and cellular processes but do not themselves cause direct harm or disease. We use them to examine how specific proteins affect the mouse's susceptibility to develop diabetes and blood vessel diseases. Information obtained from genetically altered mice can be complemented by treating the animal with a drug or infusing it with cells from another animal.

We promote type 2 diabetes in mice by feeding a high calorie and high fat diet. This leads to obesity and diabetes just like in humans. We induce type 1 diabetes by injection of a drug which damages the insulin-producing cells in the pancreas. We assess the diabetes status of mice by taking blood samples after giving insulin or glucose. We can measure energy usage, activity and metabolic rate by housing mice temporarily in a special cage.

We gain basic information on blood vessel health by measuring blood pressure with a cuff around the tail and by taking scans of mice using ultrasound, MRI, CT or laser. Blood vessels are studied in the laboratory after the animal has been humanely killed.

We gain more detailed information on blood vessels and their roles in disease in separate groups of mice in which we study particular human diseases. We study atherosclerosis (furring up of arteries) in genetically altered mice with high cholesterol levels by feeding them a high cholesterol diet. We study the response to injury of blood vessels in mice by inserting a small wire into the artery in the leg under anaesthesia or by performing surgery to place a cuff around the artery in the neck. We investigate peripheral arterial disease by tying off the main artery in the groin, so that blood flow to leg passes through the tiny branches until new blood vessels develop. We reproduce aneurysms either by applying chemicals to the main blood vessel in the abdomen during surgery, or by infusion of a drug. We study wound healing by removing small discs of skin under anaesthetic - a 'punch biopsy' - and letting them heal. New blood vessel development is either studied in the retina of new born mice killed humanely or in small plugs of gel injected under the skin in adult animals. Finally the effects of parental influences on the next generation are investigated by giving injections to pregnant mice and then studying the offspring.

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

Genetic alterations themselves affect only the molecular and cellular processes in the body but do not cause direct harm to the animal. Our research looks at how these genetic alterations affect the mouse's tendency to develop diabetes or blood vessel disease when exposed to the interventions discussed above.

As in humans, diabetes can lead to thirst and increased urine production and high fat diets can lead to an oily coat in addition to obesity. Blood sampling and blood pressure measurements lead to temporary discomfort. Ultrasound, MRI, CT and laser scans are performed under anaesthesia from which mice recover very quickly. Metabolic testing requires animals to be temporarily housed in single cases which can sometimes cause distress.

Surgical procedures, for example to injure arteries or tie-off arteries in the groin, are performed under anaesthetic from which mice recover rapidly. Mice sometimes experience temporary weakness of the leg after surgery but are fully mobile within 24 hours. Occasionally poor blood flow to the leg can lead to loss of the tips of the toes. In most cases aneurysms do not cause any symptoms. However, as in humans, aneurysms can sometimes rupture which leads to rapid death of the animal from bleeding into the abdomen. Skin punch biopsies cause pain after they are made which settles as the wounds heal.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

One of the protocols on this licence is mild severity; ten protocols are moderate and one is severe.

The following proportions of mice are expected to experience the stated severity ratings:

mild: 400 mice (4%)

moderate: 10 180 mice (96%)

severe: 20 mice (0.2%)

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

- Killed

A retrospective assessment of these predicted harms will be due by 26 October 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The influence of diabetes on the cardiovascular system is complex. Diabetes comprises elevated blood sugar levels, raised insulin levels, resistance to the effects of insulin and activation of the immune system. All of these affect the function of cells in the blood vessel wall. In obesity, many factors are released from fat deposits into the circulation which also affect blood vessels. Development of blood vessel diseases arises from the combined effects of multiple biochemical factors, fats, circulating and locally produced hormones and growth factors on cells with the blood vessel wall. The complex interactions between these processes and the communications between individual cells as vascular diseases develop means that the diseases can only be effectively studied in animals or in humans.

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

We use a wide range of non-animal approaches to address our research aims. We use tissues from humans to identify genes which contribute to diabetes and cardiovascular disease. We perform much of our research in cultured cells from blood vessels to dissect out individual genes, proteins and pathways which influence their function. We mimic the context of diabetes by culturing cells in high glucose or high fat conditions. We generate proteins in cultured cells to assess how these behave and interact with receptors. We use computer-based modelling to design molecules to mimic the effects of these proteins. Finally we conduct clinical studies in humans to investigate the effect of diabetes on clinical outcomes and interrogate genetic databases and tissue banks to identify new targets.

Why were they not suitable?

These approaches complement and inform animal-based studies but unfortunately cannot replace them. As discussed above, the complex interaction between circulating and cellular factors implicated in the development of cardiovascular disease in diabetes means that this can only be studied in an intact animal.

A retrospective assessment of replacement will be due by 26 October 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the number of animals needed for each protocol based on our experience of using these approaches in previous projects and plans to continue work in this project along with new lines of

investigation. In most cases we have based our assessment on statistical approaches to calculate the minimum number of animals to obtain significant results. However, as new genetic alterations will be studied as informed by ongoing research, we have made assumptions on future requirements based on our best assessment of the science and our previous experience.

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

We utilised available online resources such as the NC3Rs experimental design assistant to plan experiments and perform power calculations to determine sample size. These were based on knowledge of the mean values and variability of the primary outputs for each protocol based on our prior experience and on published data. We designed experiments so that multiple experimental readouts can be derived from a single animal. We use imaging when possible so that disease development can be tracked non-invasively and confirmed by tissue approaches after humane killing.

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

We work collaboratively with other researchers at our institution, so that we can share tissues between projects and avoid duplication of animal use. We optimise breeding of genetically altered animals (performed under the authority of another licence) so that breeding is fully aligned with planned experimental requirements. We use an electronic animal management system so users can track animals remotely and plan experiments to reduce waste. We keep updated with advances in scientific techniques and with ideas for reduction in animal use from the NC3Rs newsletter.

A retrospective assessment of reduction will be due by 26 October 2027

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mouse models of diabetes and cardiovascular disease. Typically, genetically altered mice will be used to study individual genes (or combinations of genes) in disease development. This approach will be supplemented by administration of drugs, viruses or cells when required to address

scientific questions. We will use a wide range of methods and models to study the full range of human vascular disease. These are described in detail in the 'Project Harms' section of this application. Our general principle is to use the model with the least likelihood of causing suffering to address the scientific question.

We have gained substantial experience of surgical techniques during the course of our previous licence. We have performed >200 surgeries for arterial wire injury, >100 for carotid artery ligation or cuff placement, and >400 for hind limb ischaemia. This has allowed us to develop a number of refinements described in the section below. We have not needed to submit any Condition 18 reports relating to surgical procedures during the term of our previous licence.

We avoid single housing of animals unless essential for scientific reasons or animal welfare. We perform surgical procedures under general anaesthesia with routine use of analgesia. Longer procedures are covered with adequate hydration, warming tables, application of eye lubrication and post-operative warming.

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

It is necessary to use a mammal to study the complex interactions involved in the development of diabetes and cardiovascular disease and to translate the findings to humans. Although certain genetic factors implicated in blood vessel growth can be studied in zebra fish, it is not possible to model type 2 diabetes and more complex vascular pathologies in fish. Because vascular pathologies typically develop over days to weeks, is not possible to study the entire process under terminal anaesthesia in mice. Adults will typically be used as this is the life stage at which the human cardiovascular diseases in which we are interested develop.

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

Surgical procedures will be performed under general anaesthesia. Animals will be recovered in a warmed chamber following longer procedures. Analgesia will be administered routinely to avoid pain developing.

We have taken the opportunity to develop a number of refinements over the last five years during the course of our previous project licence. Induction of diabetes using streptozotocin now employs a low dose regime which in our experience avoids severe hyperglycaemia and ketosis. Induction of aortic aneurysm by angiotensin II infusion employs a modified dosing regime based on our previous studies in which we reduced the dose to minimise the risk of aneurysm rupture. Experience in arterial wire injury surgery has allowed us to reduce operative time to 15-25 minutes; we use minimal contact with the neurovascular bundle and avoid disturbing the associated fat pad; we perform the arteriotomy in the saphenous branch to avoid ligating the main artery and to reduce complications such as immobility of the leg. For hindlimb ischaemia surgery, we have reduced operative time to 15-22 minutes; we avoid injuring surrounding tissues when ligating the artery; we use 8.0 suture to improve healing of the wound; we massage the leg after surgery to improve perfusion and encourage early mobilisation.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will use the following resources in planning and conducting experiments:

ARRIVE Guidelines 2.0. https://arriveguidelines.org/arrive-guidelines

PREPARE Guidelines. https://norecopa.no/prepare

NC3Rs Experimental Design Assistant. https://eda.nc3rs.org.uk/

NC3Rs guidance on blood sampling in mice. https://www.nc3rs.org.uk/3rs-resources/blood-sampling/blood-sampling-mouse

NC3Rs guidance on microsampling, including the microsampling decision aid. https://www.nc3rs.org.uk/3rs-resources/microsampling

NC3Rs Mouse Grimace Scale. https://www.nc3rs.org.uk/3rs-resources/grimace-scales/grimace-scale-mouse

NC3Rs guidance on anaesthesia. https://www.nc3rs.org.uk/3rs-resources/anaesthesia

NC3Rs Guidance on analgesia. https://www.nc3rs.org.uk/3rs-resources/analgesia

NC3Rs Guidance on handling and restraint. https://nc3rs.org.uk/3rs-resources/handling-and-restraint

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf

EFPIA/ECVAM good practice guide to the administration of substances and removal of blood, including routes and volumes. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jat.727

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

Our group will stay informed through the NC3Rs website. Relevant information, including the NC3Rs newsletter, is circulated within our institution by email to all personal and project licence holders. We will attend local events organised by our Animal Welfare and Ethical Review Committee and information sessions on NC3Rs funding streams organised by our institution's Research & Innovation Service. We will share best practice within our institution and have well developed interdisciplinary networks to facilitate this. We will hold regular local user-group meetings for project licence holders at which the group will receive updates on any changes to best practice or requirements.

A retrospective assessment of refinement will be due by 26 October 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?