Home Office

NON-TECHNICAL SUMMARY

Molecular mechanisms in cardiometabolic disease: effects of diabetes on the heart

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

Diabetes, Heart failure, Myocardial infarction, Skeletal muscle

Animal types	Life stages
Mice	adult

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 on how diabetes affects the heart after a heart attack and worsens the effects of heart failure on the body.

A retrospective assessment of these aims will be due by 24 November 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 heart failure 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. In combination with the tendency for people to live longer, there will be many more people living with the consequences of diabetes and heart failure in future.

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 heart disease and how these are worsened by diabetes. In particular we will understand the actions of insulin and related proteins within the heart and muscles 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 and discovered how they affect the body.

Our short term 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 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 heart 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: 5850

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 to study how that gene influences diabetes and its effects on the heart. 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. 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 blood vessels and the heart, we can see how insulin and its effects on those cells might influence the susceptibility to recovery from heart attacks and the development of heart failure.

Mice are amenable to studying 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 heart attacks and heart failure as well as studying the effects of treatments. We can also examine how the influence of diabetes on blood vessels affects the body's capacity to repair and heal itself after a

heart attack. Because the physical limitations experienced by people with heart failure is caused by the effects of their condition on their muscles as well as their heart, it is important that we study the influence of diabetes and heart failure on skeletal muscle.

The majority of our research is carried out in adult mice as this is the life stage at which most humans develop heart disease.

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

Most mice used in this project will be genetically altered and will be bred under a separate licence held by the applicant for breeding of genetically altered animals. 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 genes affect the mouse's susceptibility to develop diabetes and heart disease. We will compliment information obtained from genetically altered mice in some cases by treating the animal with a drug or infusing it with cells from another animal.

In many of the mice in our project we induce type 2 diabetes 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 diabetes in mice by taking blood samples after giving an injection of glucose or insulin. We can measure energy usage, activity and metabolic rate by housing mice temporarily in a special cage.

We gain more detailed information on diabetes and the heart in groups of mice in which we study particular human diseases. We assess the effects of heart attacks in mice by placing a suture around one of the coronary arteries in an operation performed under anaesthesia. This blocks the blood supply to the heart just like in a human with a heart attack. The resultant damage to the heart muscle can be assessed by ultrasound, MRI or CT scans. We can reproduce other heart muscle diseases seen in humans, such as those caused by viruses, drugs or alcohol, by infusing drugs into mice which reduce the pumping of the heart. We can also mimic the effects of high blood pressure or diabetes, which cause thickening of the heart muscle, by placing a constriction around the main artery at the outflow of the heart. All of these methods of heart injury in mice predispose to the development of heart failure and allow us to see how the body responds in the presence of diabetes.

Although we know that both diabetes and heart failure affect the way the body's muscles work, and reduce the ability to exercise, little is known about how diabetes and heart failure together affect muscles. Inability to undertake physical activities in people with heart failure can also cause a deterioration in muscles because the are not being used. To examine this in mice, we can place a tape around one of its hind legs so that mouse cannot bend its ankle. This results in the mouse not being able to use muscles in the calf and allows us to study the effects of disuse on these muscles.

We gain information on the heart and circulation by measuring blood pressure with a cuff around the tail, by taking blood samples and by taking scans of mice using ultrasound, MRI or CT. Heart tissue and muscle tissue can be studied in more detail in the laboratory after the animal has been humanely killed.

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

The genetic alterations themselves affect only the molecular and cellular processes in the body but are not expected to cause direct harm to the animal. Our research looks at how these genetic alterations affect the mouse's tendency to develop diabetes or heart disease when exposed to the experimental approaches 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 and CT 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 place a suture around a coronary artery or around the aorta, are performed under a general anaesthetic from which most mice recover rapidly. However, they are invasive procedures with up to 10% risk of mice dying during or shortly after surgery. As in humans, causing a heart attack or heart muscle damage in a mouse can lead to sudden death. The risk depends on the type of experiment, but can occur in up to 30% of mice studied. Death occurs instantly and is usually caused by rupture of the heart muscle or an abnormal electrical rhythm. Around 10% of mice develop signs of heart failure after a heart attack or heart muscle damage. Like in humans, the signs include rapid breathing and inability to exercise normally. If these persist for 24 hours the animal will be humanely killed. Taping the lower limb prevents the mouse from being able to bend the ankle joint in that leg. Mice can still move around adequately and the tape is applied for no more than two weeks.

Because we need to study how diabetes, heart disease and muscle impairment all contribute to the symptoms experienced by humans with diabetes and heart failure, some animals will be subjected to all three conditions of diabetes, heart muscle damage and limb immobilisation.

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)?

Three of the protocols in this licence are moderate severity and four are severe. However, some animals not exposed to serial optional steps may experience mild severity. Overall the following proportions of animals are expected to fall within each severity:

mild: 15%

moderate: 73%

severe: 12%

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

- Killed

A retrospective assessment of these predicted harms will be due by 24 November 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 not just elevated blood sugar levels, but in most cases raised insulin levels, resistance to the effects of insulin and activation of the immune system contribute to its effects on the body. All of these affect the function of cells in blood vessels and in the heart. In obesity, factors are released from fat deposits into the circulation which also affect the heart. The complex interactions between these various processes and the communications between individual cells as heart diseases develop means that these 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 fat depots, blood vessels, muscles and the heart 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. All of these approaches feed into the design of the experiments in this project.

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 24 November 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 for continued and new work in this project. 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 24 November 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 coronary artery ligation to induce myocardial infarction and >100 for transverse aortic constriction. This has allowed us to develop a number of refinements described in the section below.

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 vessels or heart development can be studied in zebra fish, it is not possible to model type 2 diabetes and more complex cardiovascular pathologies in fish. Because cardiac 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 cardiovascular diseases we are studying usually occur in humans.

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. We have reduced the duration of surgery for coronary artery ligation from 1.5 hours at the beginning of our experience to 35-45 minutes currently. This allows more rapid recovery from anaesthesia. We have optimised the site of the thoracotomy depending on how

proximally the coronary artery is ligated and the size of the infarct required to address scientific objectives. We access the thoracic cavity between 2nd and 3rd ribs for larger infarcts and between 3rd and 4th ribs for smaller infarcts. For transverse aortic constriction, we have reduced the duration of surgery to 25-40 minutes. We routinely employ endotracheal intubation which has been reported to improve survival. In our hands mortality in mice undergoing transverse aortic constriction with endotracheal intubation was 11.6% (compared to 20-25% in the published literature). We restrict transverse aortic constriction surgery to mice weighing 23g or more to reduce the risk of surgical mortality.

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.727s.

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 24 November 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?