



Home Office

NON-TECHNICAL SUMMARY

The biology of body weight and body composition

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

Key words

Obesity, Cachexia, Body composition, Diabetes

Animal types

Life stages

Mice

Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

To understand how body weight and body composition are controlled and how disturbances in this control lead to disease.

A retrospective assessment of these aims will be due by 10 May 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 its 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?

Understanding the processes that govern body weight is highly relevant to clinical practice as disorders that arise when these processes go wrong cause a great deal of illness and suffering. Obesity, defined as excessive storage of energy as fat, is a serious issue that drives medical conditions, with obese individuals at increased risk of developing problems with their heart, blood vessels, kidney and liver, as well as being more likely to be affected by a number of different cancers. These problems bring added personal burden to the affected individual and the healthcare support needed to treat these diseases costs a lot of money. In 2017 Public Health England reported that the UK-wide NHS costs attributable to overweight and obesity would reach £9.7 billion by 2050.

At the other extreme, cachexia is a syndrome of negative energy balance where muscle and fat mass are progressively lost. It affects over a third of all cancer patients and is strongly associated with both reduced tolerance to anti-cancer therapy and reduced survival times.

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

In undertaking these studies, we will generate new data on the biological process that control body composition. These data will combine with our on-going studies in human populations and will be presented in peer-reviewed publications and shared with colleagues in academia, medical sciences and industry.

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

In the short term the major beneficiaries will be the metabolic scientific community. We also expect our research to be of benefit to the fields of cancer biology and cardiovascular disease.

In the longer term we expect our research to be of benefit to the pharmaceutical industry, where the genes and processes we identify will provide new targets for the creation of drugs to combat body weight-associated diseases with the ultimate goal that will, even in a small way, improve human health.

Finally, we believe that work under this licence will have a role in shaping and training the next generation of researchers working on understanding how "whole body physiology", the important science of understanding how each of the multiple component parts of the body communicate to each other to allow the body as a whole to survive and thrive.

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

We will share our data and ideas with national and international collaborators and will publish our work in open access publications. We will look to share all the data we generate. We believe that it is as important to share outputs and insights that show a pathway or molecule does not have a key role just as much as it is to highlight significant insights from more novel results. We also have an active engagement with the public via lay science pieces, national newspapers and Twitter.

As our work evolves from our more historic base of understanding the causes and consequences of obesity to tackle issue related to other disorders of body compositions (such as a loss of lean and skeletal muscle mass) we believe that we can generate new knowledge relevant to human disease and ill health. Underpinning this is clarity around mechanism that control turnover of material within metabolically relevant body parts, and our recent group expansion and increased funding will enable us to explore the hormonal control of lean mass. **Further, these combined skills have helped us develop a new genetically engineered mouse line to understand a novel gut peptide that we have identified from human studies. This increased work will, we hope, be highly beneficial in open up a new field of study linking the gut with liver and lipid metabolism relevant to human disease.**

Through our involvement with Cancer Grand Challenges (CGC) initiative and our involvement with an international team of collaborators, we believe our work has potential to advance thinking around the causes of cancer cachexia. A powerful feature of the CGC team is the presence of articulate and resourced patient advocates at all of our scientific meetings. Sharing our work and our thinking with them is mutually beneficial. The patient groups get a real sense that the issues of weight loss associated with cachexia are being taken seriously by the scientific community and feel empowered to encourage patients living with cancer related weight loss to report issues and seek help from care providers.

Species and numbers of animals expected to be used

- Mice: 18,400

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.

Human metabolic disease typically comes about because of the complex cross talk and interaction between external factors (like diet, activity and environmental conditions) and internal factors such as levels of hormones in the blood and chemical signals in the brain.

We believe that mice have the necessary, very similar organ structure and hormonal systems to humans that means studying them is going to be a really informative way to improving understanding of the "chemicals of life" like sugar and fat that are knocked out of balance in human metabolic diseases like obesity and diabetes.

Mice also offer a system in which precise changes in genes and proteins of interest can be made to really focus in on the detail of how the component parts of a mechanism all link in and work together.

As the majority of human metabolic disease affects adults, most of our work will be in adult mice. However, we recognise that these diseases only emerge after many years of exposure to particular diets and lifestyles so to be able to replicate this in a model system we will need to study younger animals.

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

The majority of the work will focus on studying animals that have been designed to either make a gene not work at all ("loss of function") or work at a higher rate of activity ("gain of function"). We particularly want to know how these changes in gene function alter the responses to environmental challenges and drugs used to treat disorders of body compositions. The vast majority of the time, there are minimal, if indeed any, impacts on overall animal wellbeing and function when these genes are so targeted. On rare occasions, the loss of a gene may have more of an effect, including one which is unanticipated or not linked to the disease area under study. This looks to be the case for mice lacking LAMA4 where we have reported an increasing awareness of sudden, unanticipated death in a very small number of adult mice (less than 2%).

These procedures can be broadly grouped into 3

- Procedures needed to create the model; the genetic engineering done in the laboratory sometimes need additional delivery of other compounds to complete the process. Sometimes these additional compounds can be as simple as an antibiotic but may need to be more complex, such as a modified virus carrying genetically engineered information to alter gene expression or label a cell population. Whenever possible these reagents will be given by the oral route (either by mixing in the diet or by a tube inserted via the mouth into the stomach) but sometime may need to be delivered directly into the body by injection. These injections may be intravenous (into a vein), intraperitoneal (into the body cavity) or subcutaneous (under the skin). As we are focused on body weight, we will

frequently change the dietary intake of an animal, both in terms of constituents and amount consumed. This can mean supplying a diet that will make the animal put on weight as well as restricting food to bring about weight loss.

- Challenges to test the system; animals will be given drugs, naturally occurring hormones and biological active reagents such as antibodies, as well as naturally produced metabolites such as sugars, proteins and fats. These will mostly be delivered by injections into a vein, into a body cavity or under the skin but sometimes may require the placement under the skin of small pellet-like, devices that act as a depot for drug delivery over several weeks. We will also use modified virus that can carry genetically engineered information as delivery systems to change levels of gene expression in relevant target tissue. Because we understand that the brain has such a crucial role in controlling body weight, we will sometime need to undertake surgery to enable us to deliver drugs and hormones directly into regions of the brain that we know control how we eat or expend energy. When we do this the mouse will be under a general anaesthetic and will be unconscious. A mouse will only usually have this kind of surgery once and the procedure takes around 20 minutes. We use a specially designed operating table that enables small sterile tubes to be placed into specific regions of the brain accurately and quickly and the mouse is usually fully recovered and back to eating and drinking within 2 hours.

We will also look to better model human diseases where loss of fat and lean mass are prevalent by placing under the skin cell lines/ tumour derived material to act as an endogenous source of cell/tumour derived factors deemed likely to be drivers of changes in body composition. As these cells are derived from tumour with capacity to metastasise, mice so treated may develop lesion in other distant sites.

Animals will also encounter different environmental conditions to better understand gene- environment interactions, so crucial in the development of human disease These include diets of different composition and variable environmental temperatures. Animals will also be studied on bespoke exercise platforms such as treadmills. On occasion, we will study appetite and behaviour in a system based around drinking bottle choice. These tests are more accurate and useful tests when the animal has a drive to drink from the bottles, so in the time before these kind of tests, animals may have periods when access to water is restricted.

- Processes needed to measure and analyse the response; animals will be placed in carefully designed study chambers and imaging scanners to measure their behaviour and response to these challenges. These special study chambers look very much like the houses the animals normally live in through the week but with some simple modifications to enable, for example, sampling and measurement of the oxygen levels in the air circulating in the chamber or measurement of how much food and water the mouse has consumed. As such, the mice can easily spend several days in these chambers without undue stress. **On occasion, we will need to place a small transmitter chip under the skin or into the abdomen of the mice so we can remotely track their activity.** Similarly, when the mouse needs to have a scan to check how much fat and lean tissue they have, they will be placed in specially designed tubes made of material identical to that found in the home cage to enable them to remain still and secure and quickly have a scan without the need for sedating drugs. Whenever practical and possible, imaging and scanning will be done without anaesthesia, but on rare occasions there may be a need to use an anaesthetic agent to improve the quality of the image and data. Finally, these response measurements will sometime involve taking small amount of blood from superficial veins.

A typical experiment will involve a small series (2 or 3) of short interventions over 3-4 weeks. For example, we will give an animal a highly palatable diet for 2 weeks, treat it with a drug for a week and see what effect that had on the food intake, the body weight and the energy expenditure of the animal.

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

Most experiments proposed will lead to no more discomfort than that experienced by any rodent bred in captivity and residing in a modern animal facility.

We frequently single house animals as they progress through phenotyping protocols but the collective experience of our team and other researchers in our institute is that this is well tolerated and any adverse effects are no more than mild and transient.

Some animals will experience transient (seconds) 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. On occasion, animals may be given compounds that are recognised to produce circulating levels of hormones that are seen in acute illness. These may reduce the animals' drive to seek out and eat food in the hours after they have been given.

When given a different diet or treatment, or after placement of cell line/tumour material, some animals will gain or lose weight. This will be within closely monitored parameters that take into account other important aspects of their appearance and behaviour. This weight change will typically occur slowly over weeks. These cell lines may also lead to metastasis and tumour development in distant sites such as the lungs.

In a new protocol to investigate behavioural food aversion submitted in Autumn 2023, we plan to use a protocol involving a 2 choice water bottle model (a well validated methodology in the literature). This is a more effective test if the animals have an increased thirst drive so animals studied in this way will undergo a period of drinking water restriction for a defined period of 24hr day (typically 10-12 hrs) before returning to free access to water.

A minority of animal will also undergo surgery that will require a general anaesthetic. Inevitably, as with any operation, animals will have some discomfort in the immediate hours after the operation at the site of the incision. However, this will be minimised by administration of painkillers under instruction from a veterinarian. The general anaesthetic needed for this surgery may also make the animals less active and less hungry in the first day after the operation but we expect them to recover their appetite and vitality within 48 hours.

Animals may also undergo placement of small transmitter chips under the skin to enable remote monitoring of their movement; these are very well tolerated and do not interfere or impede normal activity or function.

Through surveillance of breeding records we have also become aware that mice deficient in LAMA4 , a scaffold protein essential for the development of fat depots have, on occasion, been found unexpectedly dead with no clear welfare concerns prior to the event. This event has occurred in less than 2% of the adult animals studied. We wish to understand more about this important protein so wish to continue to breed these animals to learn more about the biology of this important protein as it relates to metabolic disease.

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)?

Breeding protocols 90% Sub-threshold, 9.9% mild, **severe <0.1%**

Other protocols

Mild- 65%

Moderate-35%

Severe- 0.0%

August 24- No expected change in severity with amendment.

Dec 25. Addition of a "severe" breeding protocol to accommodate emerging issue around maintenance of *Lama4* null

What will happen to animals used in this project?

- Killed

A retrospective assessment of these predicted harms will be due by 10 May 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?

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 could use non-vertebrate animals which are of lesser sentience than rodents to help in our studies, none have the necessary complexity in organ structure or wider networks to adequately address the scientific questions posed. Further, the need for targeted genetic sophistication and the need to access deep internal tissue such as a brain requires a model organism system.

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

We look to use animal alternatives where possible. We use cell culture models for looking at specific mechanisms that occur in isolated cell populations. Results from these experiments can both be informed by our animal work and help us to design better animal experiments.

When looking to ask a question about the function of a gene found in the brain we have used neurons grown in a cell culture medium rather than use mouse brains. For example, through our links with colleagues who study human genetics, we have identified a number of possible genes that are linked to obesity. We also know from published work that these genes are expressed in the brain. These make them exciting candidates for future projects but before we do any work in mice, we want to be really sure they are working in the way we predict. To do that we have altered the function of these genes in neurons grown in a dish to see what happens, replacing the need to do these preliminary studies in animals

Further we have collaborated with colleagues who have gut “organoids” to test ideas on hormone action in the gut. Organoids are small, three-dimensional tissue cultures that can be grown in a dish in a lab into such an ordered pattern that they mimic a lot of the complexity of an organ, and remove the need to repeatedly collect animal tissue. Finally, with correct ethical approval and process in place, we have access to post-mortem material from humans to enable us to map out the location of genes of interest without using animals at all.

Why were they not suitable?

While cells and organoids grown in a dish are useful they can never generate the integrated data essential to this project. We cannot record from a nerve cell how fat it has become or if it is hungry, or determine how much an organoid has eaten. As such they not a sophisticated enough model platform to enable us to study the complex interplay between multiple organs that leads to metabolic disease.

A retrospective assessment of replacement will be due by 10 May 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?

These numbers are largely based on the level of work over the previous two licenses and the amount of funding we have in place and expect to use to fulfil our aims and objectives.

In an amendment made in Autumn 2023, the total number used had been amended because of an increase in anticipated activity in protocol 7 (increase of 1000). This reflects the increase in activity and the increase in group size that is needed to successfully pursue our emerging observations on the effects of GDF15 being ameliorated by antecedent levels and to match the needs of our expanding work on targeting the MC3R system. This work is fully funded through our recent Cancer Grand Challenge award (see "Funding" section below).

We have also added an amendment in Summer 2024. We have started new work on determining the hormonal link between protein sensing and lean /muscle mass accrual and developed a second body of work defining a novel mechanism linking dietary makeup with how and where lipid and cholesterol are deposited in the body. This has particular relevance to the liver and as such has wider ramifications for metabolic health.

With this funded expansion in the group (MRC Metabolic Disease Unit) we need to undertake more experiments where mice are fed an altered diet. We did consider accommodating this expansion in other existing protocols which include the ability to supply and altered diet, but feel using this simpler protocol to be the better way to answer our current questions without having to add additional harm.

As our science has evolved over this licence, we have developed new interests in molecules relevant to human metabolic disease that fit into our overall aims. These include a range of molecules made by the liver which we think have key roles in sensing and responding to changes in lean mass and protein content of the body and signalling pathways in the liver that strongly influence the types and location of lipid deposition in the body. This focus means we wish to pursue more studies where dietary changes, drug treatment and lipid biology are the focus and as a result will undertake fewer studies where CNS surgery and exercise are necessary. As such in November 25, based on current levels of scientific activity, reagent availability, current staffing and unit occupancy we have refined the numbers to be used on relevant protocols to better match this scientific refocusing . Across these changes we do not anticipate using more animals in total overall in the whole project licence.

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

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 will allow other trained researchers to work with the colonies in our unit rather than moving mice, reducing the number of mice that are both bred and transported.

Studies will be of appropriate size to detect significance, with animals randomly assigned to matched study groups and, whenever possible and practicable, investigators blinded to the nature of interventions. Protocols will include a series of analyses and steps on a single animal, rather than single analyses on multiple animals. We aim to balance impact upon an individual animal with scientific output but reason that this approach significantly reduces the number of animals used.

We think carefully about which other organs researchers in the laboratory can work on, such as heart and adipose tissue, and harvest these from the same animals. This reduces the numbers of animals used overall, increases the amount of data obtained from a single animal and allows us to examine links between different tissues/organs by combining data from the same animals, thereby enhancing the quality of the information produced.

Control groups are essential cohorts to run alongside intervention studies. To facilitate better study design and clarity of purpose, an "either/or" step is the first mandatory step of protocols 5 and 6 to enable a standard chow group to run alongside an intervention group.

As the work on the licence continues to evolve, we continue to look to reduce burden and refine design. For example, in the Amendment of Nov25, we describe how using an implanted cell line as a biological peptide delivery system removes the need for multiple daily injections and the ability to implant a telemetry probe increases the depth of data generated from an animal, with this animal being studied in a less stressful, more naturalistic enriched environment.

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

We use pilot studies for any new agent we are investigating. These are used to check the safety of drugs in a small number of animals before using them in a larger cohort but also give a real world output on effect size to design the larger study.

We routinely freeze down and store either eggs or sperm from all of the different colonies of mice we study. This means we only breed animals when we need them for experiments, rather than having to maintain a permanent colony for use in potential future experiments.

We collaborate with other groups across the UK, Europe and the USA and share samples we have banked to these collaborators for their own purposes, maximising the benefit from previously conducted studies.

We are also aware that we live in an age when more and more experimental data are placed in open access platforms that are easily accessible on-line. We continue to work closely with our experts in information technology to look through these large data sets to find information that will actively shape our experimental design.

The ability to more frequently and non-invasively image evolving body composition over time also goes some way to avoiding the need to cull cohorts of mice for tissue analysis at serial time points; this more detailed serial analysis of an individual mouse aims to reduce total numbers used. **This also applies to our plan to use remote telemetry chips to enable more detailed activity data to be recorded from each individual mouse.**

A retrospective assessment of reduction will be due by 10 May 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 mice.

We will typically use a mixture of wild-type, genetically altered and naturally occurring genetic mutant animals. Genetically altered animals are those that are carrying a specific and deliberate change in their genetic code that has been engineered to be there through laboratory manipulation of DNA.

We will use altered diets to change body weight. These are formulated to be accessible and palatable to mice.

In terms of the tests we carry out to measure circulating chemicals, either in the resting background state or after the system has been "switched on" with a challenge, we have refined protocols which deliver the smallest volume and require the smallest sample sizes possible to generate meaningful data.

In an amendment in Autumn 2023, we have also reframed "glucose tolerance testing" to cover "dynamic metabolic testing". The ability to sequentially monitor circulating glucose after a bolus of insulin or glucose is a trusted and well validated methodology. We now plan to finesse this by adding the ability to analyse other relevant metabolites (for example, amino acids and fatty acids) in the same way. The impact on the animal remains unchanged but the depth of data generated is increased.

In Autumn 2023 we also took ownership of a stable isotope analyser to expand our phenotyping capabilities. Using non-radioactive isotopes coupled with a system that can monitor non-invasively intermediate metabolism in a live animal is a great leap forward. It enables greater data acquisition with only a mild and transient burden (the administration of the reagent) on the animal under study. As the system is based on analysis of exhaled carbon dioxide rather than need to access circulating blood or tissue, this removes the need to bleed and/or cull the animal to measure the isotope flux.

Our work within the Cancer Grand Challenges consortium has enabled us to bring our expertise in body weight phenotyping to focus in on the unmet need of cancer cachexia, a devastating disorder affecting the human host. To better model the tumour derived factors causing this disease of body composition, we will place material (eg. cell lines, tumour tissue) into mice, with the purpose of phenotyping the effect on body composition of tumour-derived factors. **Again, using cell lines as an endogenous biological producer of relevant circulating factors (a "biological minipump") is a refined way to avoid days of repeated injection of substance.**

Imaging and calorimetry systems are widely used apparatus that have been continually refined and redesigned over years to minimise stress. Calorimetry systems are cages plumbed in to closed circuit

gas analyser systems which enable measurement of what gas a mouse had consumed and what gas a mouse has produced. In doing so, this enables a calculation to be made of how much energy that mouse has used up. While previous calorimetry systems had rather barren grid floors, we are pleased that we have moved some way from this system. Refinement in design and material has now made it possible to have a calorimetry system (e.g. Promethion system) based around the existing base floor unit of the home cage, where the technology and sensors are embedded in a roof unit that can be used in a mobile and modular fashion with less environment disruption to the animal under study. In doing so, this greatly facilitates a longer period of study that greatly helps eliminate concerns around data impacted by acclimatisation and stress artefact.

Environmental temperature is a very potent regulator of metabolism, with effects in particular on food intake and energy expenditure. The temperature range we have chosen is invariably well tolerated by mice, with temperatures closer to thermoneutrality often considered less stressful to animals than standard housing temperature.

Animals will also be studied on bespoke exercise platforms like treadmills, which enable the mice to run freely without need for any external stimulus. **They will also be studied in a bespoke enhanced home cage environment that fosters usual social behaviour in a naturalised and enriched manner.**

To more accurately record food intake and metabolism, we will sometimes need to single-house mice. In the past there have been concerns that this is stressful, but we will minimise the time each animal is housed alone and, where possible, animals will be re-housed in groups with their original cage-mates following a period of single-housing. In addition to shelters, nest boxes and nesting material, tubes to act as hiding tunnels, shredding toys and wooden chewing toys for animals to gnaw upon will also be supplied. These will be modified as necessary to accommodate head placed cannula in any animal that has undergone surgery and had an indwelling cannula sited.

Finally, we have a number of specialised techniques that we will perform at the end of the experiment on terminally anaesthetised animals to minimise their pain suffering and distress. Animals that are under "terminal anaesthesia" remain in a state of deep sleep and unconsciousness throughout until they are humanely killed at the end of the experiment.

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

We use less sentient animals where possible and our research group has used flies to study specific research questions.

However, as our interest is in modelling human disease states, a mammalian system is the most suitable.

We do use terminally anaesthetised animals for some procedures, but under such conditions we cannot measure processes such as food intake and energy expenditure that can only be measured in live animals over days.

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

We have made several refinements to our techniques over the past years.

Specifically;

More prolonged period of acclimatisation in an enriched environment with “same-user handling” in period prior to studies where stress is anticipated and where stress will affect data output. This has been particularly useful in studies of the stress hormone corticosterone where, without a period beforehand where the animals are used to being handled, the stress signal from the test itself may have swamped the true biological signal. Whenever possible, mice will be housed in social groups.

For calorimetry data we have taken advantage of in-house, mathematics expertise existing within our institute to factor in the inevitable change in body weight seen in the study period. This improves the quality of the data and removes the need for the animals to undergo a repeat period in the calorimetry system. The development in technology towards calorimetry systems that are based around “home cage” units has gone a long way to minimise stress and variation in this analysis.

In terms of non-invasive imaging, the increasing sophistication and ease of operation of devices such as the ECHO MRI that removes need for anaesthesia means that there is a very transient additive burden on the animal. Although there are still some scanning rigs that require anaesthesia and we do plan to continue to work with our imaging core to maximise use of evolving technology, over the last 3 years all of our body composition scans have been done without anaesthesia and daily scanning over a longer time period is now commonplace in our facility, greatly adding to the depth of phenotypic data available from a single animal. In a similar manner, the ability to place telemetry probes will mean we can collect richer data from a refined, more naturalised and enriched cage environment.

We continue to look to recheck and revise on the formulation and dosing of reagents and substance administered. For example, we have recently moved over to using a long acting form of GDF15 (coupled to an FC portion of an antibody) in place of repeat multiple injections. One single injection of an FC-GDF15 can take the place of a week of twice daily injection. Similarly, using a single injection of an engineered adenovirus carrying a gene of interest enables endogenous production of a peptide under study and is more effective and less invasive than delivery through minipump or multiple daily injection. As a result of these refinements, we have reframed and renamed the common protocol step where a reagent or substance is given.

In looking to find better models of cachexia related to malignancy, we consider using implantation of cellular material capable of continuous, endogenous production of factors that directly or indirectly impact on body composition to be a more refined model of the human disease state that removes need for repeated injection.

For food intake studies we have made our own dishes to capture spillage and made measurement of food intake a much faster and accurate process, maximising data yield from the time animals spend single-housed. Whenever we are using any drug that we wish the animal to take by mouth, we will work with our teams in the animal units to introduce appealing and attractive flavouring (like Strawberry Nesquik) to be delivered alongside the drug.

We will look to use close observation of both body weight and body condition scoring to get a more complete readout of an animal's situation to enable us to detect problems early and avoid harm. The body condition scoring system is a simple, rapid and noninvasive method for assessing health status

and wellbeing. This close observation of animals will be particularly relevant to a line we are studying - *Lama4* null mice- for the purposes of gaining insight into fat cell biology. The mice also look to have a potential risk of developing a subtle movement disorder, especially later in life, which may be a precursor to deterioration later on, including unexpected sudden death. We plan, in addition to general welfare checks and body condition scores, to undertake a simple tail lift while front paws are still on floor to look for atypical or uncoordinated movements of the hind limbs. Further we will look to undertake details tissue study for *ex vivo* analysis of tissue at monthly intervals to determine if there are early histological signs of disease in muscle and cardiac tissue (sites known to be connected to LAMA4 biology). This combined with the body phenotyping will help build a better predictive picture to identify signs of ill health before more significant events. Finally, we will look to focus our phenotyping on mice that are younger than 6 months to minimise chances of adverse features developing.

In any post-operative period we will pay close attention to ensuring the environment meets the needs of the animal by the addition of a heated environment, post-operative bedding and more palatable mashed food.

In conditioned aversion testing, a period of water deprivation before the data acquisition phase shortens the overall testing period and increases the robustness of the test to be able to generate useful data. The minimum period of deprivation needed will be arrived at using pilot studies of varying time lengths of deprivation, with the shortest possible time taken forward into larger studies. Further, we have commissioned our medical engineering department to build a customised water bottle apparatus for this assay to enable studies to occur in a familiar home cage environment.

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

Laboratory Animal Science Association (LASA) guiding principles documents of aseptic technique (https://www.lasa.co.uk/current_publications/)

ARRIVE (Animal Research: Reporting of In Vivo Experiment) guidelines for preparing papers for publication (<https://www.nc3rs.org.uk/arrive-guidelines>)

PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines for planning animal research and testing (<https://www.ncbi.nlm.nih.gov/pubmed/28771074>)- used for planning our experiments

Smith *et al.* Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. *Lab Animal* 2018 Feb;52(1_suppl):5-57. doi: 10.1177/0023677217744587.

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

Our institution has a regular advisory board meeting to facilitate meaningful dialogue across the license holder, researchers and technicians. Through these meetings we are able to find out about latest practical guidance from relevant national bodies such as the LASA (Laboratory Animal Science Association) and the RSPCA (Royal Society for the Prevention of Cruelty to Animals).

Accessing NC3R website (<https://www.nc3rs.org.uk>) as a resource for guidelines, practical information, links to publications and training materials.

Advances in the 3Rs will be disseminated to those operating under this licence through the weekly laboratory meetings.

A retrospective assessment of refinement will be due by 10 May 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?