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NON-TECHNICAL SUMMARY

Interactions between metabolism and epigenetic control in ageing health

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

metabolism, epigenetics, ageing

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

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?

How genes are organised and regulated in cells can depend upon metabolites – small molecules present in our cells – that come from our diets. We want to study how these connections change during the lifetime and affect resilience of tissues, and whether interventions can be made to improve metabolic and epigenetic health in ageing.

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?

Our genes are wrapped by proteins to form chromatin which helps condense the DNA in the cell nucleus and enables genes to be controlled properly during development and in different tissues. These normal processes are often referred to as epigenetic control of genes. Metabolites – small molecules present in our cells that ultimately come from our diet – are needed to allow different chromatin states to be adopted - influencing the availability of genes for expression. This is important as stem cells differentiate to create defined cell types. But we do not understand how the availability of the metabolites influencing chromatin processes is controlled, as the same metabolites are also used in many other processes in cells. We believe linkages between metabolites and chromatin change as we age, or in response to poor nutrition or food excess, and may contribute to how tissues lose proper function with age. We should like to test whether we can control metabolite availability to benefit ageing – we know we can do this in simple organisms, such as yeast, using cellular pathways that also exist in mammals. This could provide a rationale for diet interventions that could promote ageing health.

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

The key outputs will be new understanding of how chromatin states critical in regulating gene expression are controlled by metabolites, and how this balance changes over the life-course, with impacts on tissue function, metabolic and ageing health. These findings will translate firstly into peer-reviewed publications, but they will also inform public engagement and dialogue work we plan to undertake. In addition, beneficial metabolic interventions we discover may be protected as intellectual property.

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

(i) The academic scientific community, particularly in relation to epigenetics and metabolism. Our research will provide new, fundamental understanding of the interface between cellular metabolites and chromatin mechanisms, and how these linkages change with age. Our outputs will also contribute to future studies: new datasets and new genetically-altered (GA) strains will be generated and made available to other users to advance future research.

(ii) Funders, in particular the BBSRC. Our research underpins the delivery of a strategic priority of the funder "*Bioscience for an integrated understanding of health*", including "*to enable new mechanistic understanding of key biological mechanisms underpinning health including the biological basis of ageing*".

(iii) Clinicians working on metabolic disorders. Our outputs will help provide scientific rationale for the influences of diet and lifestyle on ageing health.

(iv) Policy makers and the general public. Benefits will include increased knowledge, understanding and awareness of the importance of epigenetics and metabolism, including the impact of diet on long-term health, and its potential social and economic relevance.

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

We have many mechanisms in place to do this in addition to the immediate academic routes of presentations at international conferences, publishing research papers in open-access journals and making datasets, new reagents and GA mouse strains openly available. The Institute has an expertly-qualified Knowledge Exchange and Commercialisation team, which has an excellent record in supporting Institute researchers in finding commercial partnerships to translate findings of potential translational significance. The Institute also has dedicated Public Engagement and Communications teams, which have an active programme of public engagement events and contribution to policy initiatives, in particular to provide expert advice around potential interventions targeting ageing. And we expect to build on and develop new academic collaborations based on our capabilities and new knowledge.

Species and numbers of animals expected to be used

- Mice: 12,750

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.

The aims of the project are to understand the linkages between metabolites and chromatin mechanisms, and how these change in ageing to result in impaired metabolic health relevant to human health. It is necessary to undertake this in an animal model, because cell models do not adequately recapitulate changes that occur in ageing. We have chosen mouse as our animal model, because it possesses many metabolic characteristics relevant to chromatin regulation similar to humans and, for a mammalian species, has an ageing lifespan that can be studied within practical timescales. In addition, we are able to benefit from existing genetically altered (GA) strains for refinements such as tissue-specific control of the genetic modifications we may introduce.

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

The most common procedure in this project is breeding and maintenance to produce adult mice that will be killed via approved Schedule 1 to supply cells and tissue for the aims described in this project. Some mice will be kept until they are aged to ~28-months of age. Breeding will include genetically altered (GA) mouse strains, and we shall also be generating new GA strains, including the use of highly-refined genetic modifications that will selectively affect genes in tissues of interest, such as fat cells or the liver.

Small numbers of mice will be fed altered diets, such as high-fat diets or diets with reduced amino acids, to evaluate the effect of diets on how the genes we are interested in are controlled. These diets are not expected to cause distress but may result in obesity, or minor weight loss. Some mice will have blood sampled to measure glucose and other metabolites; these mice are not expected to experience more than mild and transient discomfort from blood sampling.

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

Many of the genetic alterations in mice we breed are not expected to change function of genes (for example, they are tags to help us isolate cells of particular interest to high purity); breeding of these strains is not expected to have adverse effects.

For genetic alterations that we suspect could cause a harmful effect if present in the whole animal, we shall use genetic methods to limit the alteration to tissues of interest (e.g., fat cells, liver) thereby minimising the risk of adverse effects.

Some genetic alterations may alter metabolism in mice, leading to possible longer-term conditions such as obesity or diabetes; these conditions will be monitored to avoid development of harmful outcomes.

Mice fed altered diets, e.g., high-fat diet over a period of two to three months are expected to become mildly obese and diabetic, but these will be monitored to avoid development of harmful side-effects.

For mice that undergo surgery, mostly for transferring embryos, the duration of anaesthesia and surgery is very short and the animals are expected to make a full and unremarkable recovery, although analgesia will be administered to mitigate short-lived pain.

Mice being administered tracer or labelling substances, which may be done by injection or orally, are not anticipated to experience any adverse effects, beyond transient discomfort due to the injections.

Mice administered insulin or substances that mimic adrenalin action may experience short-term reduction in blood glucose which, in rare cases, could cause altered behaviour or fitting. Mice would be continually monitored and if such effects occur they would be treated by injecting glucose to restore normal blood sugar levels.

For mice that are allowed to reach advanced age (>18 months), we do not anticipate specific impacts or adverse effects in mice ageing healthily. Animals that begin to show irreversible ill health, such as sustained weight loss, will be killed because we are interested in healthy ageing and not the ageing of diseased or unhealthy animals.

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)?

The expected overall severity of this project licence is Mild, with fewer than 10% of animals expected to experience a maximum severity of Moderate.

Procedures involved in the generation of new genetically altered mouse strains: 50% moderate; 50% mild

Breeding/maintenance genetically altered mouse strains: 5% moderate; 10% mild; 85% sub-threshold

Metabolic and diet studies in mice: 5% moderate; 95% mild

Ageing of mouse strains: 5% moderate; 95% mild

Administration of antibiotics to mice: 5% moderate; 95% mild

Administration of chromatin-modifying agents to mice: 5% moderate; 95% mild

Administration of β3-adrenergic inhibitors to mice: 100% moderate

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

- Killed

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?

We are seeking to understand how the availability of metabolites critical for chromatin regulation, and thus for fidelity of cell state and function, changes with age and may contribute to loss of tissue resilience that accompanies ageing. We are also planning to identify interventions that could mitigate these age-related declines. We need to conduct some of this work in whole animals, because ageing cannot adequately be modelled in cell-based systems.

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

We are able to perform parts of our work in *in vitro* mammalian cell systems – those that investigate how our chosen genetic modifications alter metabolic pathways and chromatin processes outside of the context of ageing. These *in vitro* systems help refine the experimental approaches before applying them to mouse models.

Why were they not suitable?

The main limitations of mammalian cell culture systems are that they cannot properly recapitulate the process of ageing, nor faithfully reflect responses to interventions such as altered diet. In addition, metabolic interventions within specific cells could have effects systematically or on other organ systems, and these potentially beneficial or adverse effects would be missed in cultured cells that represent single specialised cell types.

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?

From experience of similar experimental designs in previous projects. With advice from the Institute statistician in relation to the minimum number of animals (data points) necessary to achieve statistically robust results in any procedure with a quantifiable outcome. Annual Return of Procedures data from our current licence to estimate the number of animals that we will need to use for breeding.

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

For the design of quantitative studies we generally follow the ARRIVE guidelines and can use power analysis to determine sample sizes. We typically use a significance level of 5% and a power of 80%, estimating standard deviation from pilot experiments if necessary or from comparable prior studies. We include advice from the Institute statistician in addition to making use of online tools, such as the NC3Rs' Experimental Design Assistant.

We can reduce animal numbers needed by making multiple measures from the same animal or sample, wherever possible. For example, current protocols for molecular profiling of tissues *ex vivo* enable us to obtain measures of gene expression and chromatin state in the same assay; metabolomic analyses can profile >90 metabolite species in a single sample.

As well as reducing the total number of samples, thus animals, needed to obtain these measures, obtaining multiple data from the same sample represents a refinement in experimental design.

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

Use of power calculations for optimized animal group sizes based on comparable data from previous experiments and advice from the Institute statistician.

Minimising inter-group variability using controls of matching age, sex and genetic background.

Cryopreservation of strains when no longer required.

Use of colony management software that helps avoid overproduction.

Collection and freezing of tissues post-mortem beyond those needed for our own experiments, to make available to other researchers who may be interested in similar questions (e.g., aged tissues).

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 have chosen mouse as our animal model, because it possesses many metabolic characteristics relevant to chromatin regulation similar to humans and, for a mammalian species, has an ageing lifespan that can be studied within practical timescales.

Some mice will be allowed to grow old (>18 months), because we are studying how linkages between metabolism and chromatin regulation change with age: animal house staff are trained and experienced in the recognition of signs of ill health that could be found in ageing animals and we would expect to humanely kill animals that show signs of suffering that is greater than minor and transient or in any way compromises normal behaviour.

Some mice will be fed altered diets and/or challenged metabolically, for example, by injection of glucose or adrenalin mimics: this will be done to test how new genetic modifications of our genes of interest alter metabolic pathways or lead to changes in function of metabolically relevant tissues.

Some mice will be administered substances by injection or oral gavage for metabolic tracing to test how they are incorporated into chromatin; the substances and routes of administration are not expected to cause anything other than transient discomfort.

Some mice will undergo surgery for implanting embryos to generate new genetically altered strains. The duration of anaesthesia and surgery is very short and not expected to cause more than short-lived pain, which can be controlled with analgesia. Non-surgical embryo transfer methods will be used where the success rate matches that of surgical embryo transfer methods or is sufficient for the aims of the experiment.

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

The focus of our study is how metabolic and chromatin integrity changes during the life-course and into the aged mammal, so we need to maintain mice over a range of adult stages. In addition, we are studying the linkages between metabolites and chromatin mechanisms particularly in tissues (fat) that are metabolically relevant in warm-blooded mammals rather than cold-blooded vertebrates or less sentient animals that are physiologically less similar to human physiology.

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

Harm to animals is minimised by using sterile conditions, anaesthetics in surgical procedures; humane methods of killing; regular surveillance to quickly identify deviation from health; and by targeting possibly harmful genetic mutations to the cells of interest (e.g., liver, fat tissues) to avoid the possibility of whole-animal suffering.

Housing, husbandry and care conditions are provided by a dedicated Biological Support Unit (BSU), staffed by highly-trained animal technicians and overseen by experienced supervisors and NACWOs. The BSU enjoys permanent and expert veterinary cover.

If, in rare circumstances, an animal has an unexpectedly severe response to a procedure or diet, or where an infection develops, treatment is given where possible and, if necessary, the animal is humanely killed.

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

Planning and conduct of the project is informed by the ARRIVE and PREPARE guidelines.

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

We keep fully aware of developments in cell-based and organoid systems that could REPLACE some of the animal use and which could be of benefit to the aims of the project through the published literature, conference attendance and interactions with science groups working on related questions, and would adopt them where we can, if they prove reproducible and representative of the *in vivo* situation. We also remain in regular communication with the Institute's animal facility staff about husbandry and procedural developments that could provide gains in REDUCTION and REFINEMENT. The animal unit maintains an up-to-date online library of 3Rs resources.