G. NON TECHNICAL SUMMARY (NTS)

Project title: Nutritional programming of metabolic disease
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES
(b) translational or applied research with one of the following aims:
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants: YES
   (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants: YES
   (iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes: NO

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b): NO
(d) protection of the natural environment in the interests of the health or welfare of man or animals: NO
(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work: NO
(f) higher education or training for the acquisition, maintenance or improvement of vocational skills: NO
(g) forensic inquiries: NO

Keywords:
Suboptimal nutrition, pregnancy, early life, programming, adult health

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):
Our overall purpose is to identify the mechanisms leading to adult disease, secondary to suboptimal maternal nutrition during pregnancy and/or lactation, and to investigate the efficacy of interventions that are potentially translatable to humans.

The key questions are:

1. How does suboptimal gestational and/or lactational nutrition affect maternal and offspring health and what are the underlying mechanisms?
   - What are the key maternal factors (e.g. hyperinsulinemia, hypertension) in an obese mother that program metabolic fitness, cardiac dysfunction, energy balance in offspring?
   - As proof of principle, can we mimic these programming effects (e.g. insulin) by introducing these maternal factors directly into the fetuses?

2. What are the effects of pharmacological and/or exercise intervention to the mother, and the metabolic health of her offspring?
   - Does perinatal maternal exercise in an obese mother improve her metabolic and cardiovascular fitness?
   - Does perinatal pharmacological treatment improve her metabolic and cardiovascular fitness?
fitness?

3. What are the effects of dietary/pharmacological intervention in the offspring on the programmed effects on metabolic health?
   - Does perinatal maternal exercise in an obese mother improve metabolic and cardiovascular fitness of her offspring?
   - Does perinatal pharmacological treatment in an obese mother improve metabolic and cardiovascular fitness of her offspring?

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?
Lifestyle (increased physical exercise) intervention to an obese mother around the time of pregnancy is likely to improve her metabolic fitness and the long-term health of her offspring. This is an important translational message.

What types and approximate numbers of animals do you expect to use and over what period of time?
Over the 5-year period of this project, we expect to use no more than 6,900 adult and 7,500 neonate Rats; 11,600 adult and 10,400 neonate Mice.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?
The large proportion of animals to be used in this licence (81.9% of the adult rats and 87.5% of the adult mice) will experience no adverse effects. Female animals fed differing diets, with or without exercise or pharmacological intervention, will then be paired with a male. Both mothers and offspring health will be monitored throughout adult life using longitudinal assessments of a) non-invasive cardiovascular imaging techniques, sometimes with a general anaesthetic to negate stress b) metabolic testing involving All animals will be killed humanely at the end of the experiment. 15.2% of the adult rats and 10.8% of the adult mice will undergo surgery under appropriate general anaesthesia. Following surgery, they will experience minor discomfort with itching around the wound stitches. This will be managed with appropriate analgesia and antibiotics. All animals will be killed humanely at the end of the experiment. 2.9% of the adult rats and 1.7% of the adult mice will undergo surgery under general anaesthetic throughout the experiment and at the end of the experiment, they will be killed by an anaesthetic overdose.

Application of the 3Rs
Replacement:
Although experiments in cultured cells can provide a lot of useful information on specific workings at a cellular level, we need to study how different cell populations behave and interact as part of a complex environment in a living animal. Each tissue type e.g. brain, fat tissue, heart, working muscle or liver, are themselves made up of different cell populations (including stem cells which go on to divide and mature into fully functioning adult cells). The different tissue types send out and respond differently to the signals present in the peripheral blood system such as occurs in the whole living animal. This level of complexity cannot be attained in cell culture based experiments.

Our previous work has used the strategy of identifying specific mechanisms due to suboptimal maternal environment in animals which lead to cardio-metabolic disease in the offspring, and then using this information to guide parallel human studies (e.g. in Danish low birth-weight men). This successful strategy clearly underlines the value and justification for our work using animals, which has significant parallels with the human pathophysiology. We will where possible, use a similar approach to translate our observations in animal models into humans by working on human serum and biopsy material and we will continue to use a forward and back translation approach.
Reduction:
It is important to note that as the environmental stimulus is made to the mother, it is the mother which becomes the statistical unit for all our studies, and this is therefore reflected in our animal numbers as we are constrained to using only 1 offspring in each litter for any given outcome. We will collect all tissues and organs at post mortem—even ones that are not required at the time for any particular study. These include brain, heart, aorta, lungs, liver, pancreas, fore-gut, hind-gut, kidneys, intraperitoneal fat, retroperitoneal fat, gonadal fat, vastus lateralis & biceps femoris muscle, brown fat, bones and testes or ovaries. This extensive bank of tissues allows us to follow up several lines of disease pathology, so that we and other groups through internal and international collaboration, are able to facilitate later studies without the need for additional numbers of animals, thus significantly reducing the need to use more animals.

Where possible we will follow up observational studies in the animal with non-animal cell systems to gain mechanistic insight into specific cell signaling pathways. These studies will help define the specific pathways involved and thus inform specific intervention, resulting in a reduction in the number of animals and a high degree of refinement to the proposed intervention models. For example, we observed that maternal obesity "programmed" a loss of IRS1 (an insulin signalling molecule) in the fat tissue of the offspring of obese mouse dams. In the same tissues, we also observed a gain in the expression of a small RNA molecule (microRNA) known to negatively regulate IRS1 protein levels. In order to investigate if these programmed changes could be replicated in a cell system, we obtained precursor cells from the fat tissue of these mice and grew them into mature adipocytes in vitro. This experiment showed us that despite being grown and outside the animal, these precursor cells carried the information encoding the programmed phenotype observed in the mature fat cells of the animal. This strategy will allow us to reduce animal numbers further by adopting a cell system widely used in studies of regulation of fat metabolism for our more complex studies. We will also couple the use of this cell system with a global approach to identify other proteins regulated by the overexpression of any microRNA in a non-biased manner and without a priori bioinformatics prediction.

We will use non-invasive echocardiography with recovery anaesthesia to monitor cardiac function longitudinally, in the same animal that reduces the number of animals required. Isoflurane is very well tolerated in every animal as we maintain anaesthesia for no longer than 20 minutes and recovery is quick (under 1 minute). This provides substantial gain in power and data quality and robustness thus reducing animal numbers.

Refinement:
Females from our breeding colony will be randomised (by animal technicians blinded to the study) to receive experimental diets and or interventions. At sexual maturity, they will be mated with male studs that are refreshed every 6 months to optimise breeding and minimise ageing paternal effects on the offspring. Offspring are again randomised (by technicians blinded to the study) to be weaned onto control or obeseogenic diets with or without pharmaceutical intervention. Where possible, investigators carrying out cardiometabolic measurements will be blinded to the experimental groups.

In our exercise intervention studies, we apply the knowledge that rodents are nocturnal and therefore they respond better to exercise training at the beginning of their wake cycle (which is after 6pm in the evening when the lights are out). Our researchers therefore go in to train the animals in the dark with a red headlamp to minimise disturbances to their circadian rhythm.

We use non-invasive TDNMR that does not require anaesthesia to measure body composition of mothers and offspring. As this allows longitudinal body composition to be measured in the same
animal, it also reduces the number of animals required. We combine this data with other repeated longitudinal measures such as non-invasive tail cuff blood pressure and Echocardiography. Finally, at post-mortem, we take blood to measure metabolites and tissues for molecular studies. All the data can be correlated and then also compared to aged controls to identify if there is an advanced aging phenotype. From this, we can identify markers at the cellular level indicative of ageing, which enables us to assess the effects of early nutrition on life span without the need for maintaining mice and rats for their full lifespan. During the next 5 years, we hope to acquire access to more advanced imaging equipment that would allow in-utero measurements and measurement of ECG in conscious animals.