Home Office

NON-TECHNICAL SUMMARY

The role of adiponectin in equine endocrinopathic laminitis

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

laminitis, adiponectin, insulin, endocrinopathy

Animal types	Life stages
Ponies	adult
Horses	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.

- Uses cats, dogs or equidae

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 further elucidate the role of the adipose tissue derived hormone adiponectin in the pathogenesis of equine endocrinopathic laminitis.

A retrospective assessment of these aims will be due by 16 June 2026

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?

Laminitis is a painful condition of the equine foot that affects approximately 4% of the horse and pony population in the UK and worldwide. In addition, it is frequently recurrent, with up to 70% of animals suffering from repeated episodes. There are three forms of laminitis, namely sepsis-associated, endocrinopathic and supporting limb laminitis. Endocrinopathic laminitis is the commonest form, accounting for up to 90% of cases and encompasses laminitis associated with the endocrine (hormone) diseases equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID). The key feature of EMS is insulin dysregulation (ID), which is abnormal insulin metabolism in response to a normal physiologic process, such as eating. In horses, this manifests as high blood insulin concentrations (hyperinsulinaemia) and/or an excessive insulin response to ingested carbohydrate and/or resistance to insulin at the level of the tissues. Additional features include obesity, high blood fat concentrations (hypertriglyceridaemia) and abnormal fat tissue metabolism (adipose dysregulation) manifesting as abnormal plasma adipokine (hormones produced by fat tissue) concentrations, including low circulating concentrations of the hormone adiponectin (hypoadiponectinaemia). It is well established that high blood insulin concentrations for a prolonged perios (48-72 hours) can induce laminitis, but the underlying mechanism remains unclear. Current research has focused on insulin binding to and inappropriately stimulating the receptors for the hormone insulin-like growth factor-1 (IGF-1) which are found in the equine foot. Adiponectin has anti-inflammatory and insulin-sensitising actions and we have previously demonstrated that low circulating adiponectin concentrations, as well as high blood insulin concentrations, is a risk factor for endocrinopathic laminitis. In other species, the

adiponectin and insulin signaling pathways within cells converge at the level of the adaptor protein APPL1 and there is emerging evidence of cross talk between adiponectin via its receptors and both the insulin and IGF-1 receptors, resulting in increased and decreased signaling respectively. This project will firstly investigate the effect of high blood insulin concentrations, induction of tissue insulin resistance (using corticosteroids) and obesity (via pasture-induced weight gain) on circulating adiponectin concentrations *in vivo*. Human metabolic syndrome (HMS) is very similar to equine metabolic syndrome in terms of the metabolic alternations that occur and it is associated with an increase risk of certain cardiovascular diseases. Dietary manipulation and pharmacologic agents are used to increase circulating adiponectin concentrations in people with HMS and this in turn reduces the associated cardiovascular disease risk. Thus, this project will also determine whether similar approaches can be used in EMS. The effect of weight loss with or without dietary supplementation and/or pharmacologic agents on circulating adiponectin concentrations will be evaluated. Potential pharmacologic agents will first be screened *in vitro* through evaluation of their effects on equine fat tissue (adipocyte) adiponectin production.

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

This project seeks to further elucidate the role played by adiponectin in endocrinopathic laminitis and to identify potential pharmacologic agents and management interventions that will increase circulating adiponectin concentrations. This in turn may reduce the risk of endocrinopathic laminitis in high risk animals. This new information will be disseminated in the form of presentations at suitable equine veterinary and research conferences and publications in suitable journals.

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

The project has a potential beneficial welfare impact for horses and ponies worldwide and an economic impact for their owners through reduced veterinary expenditure and athletic performance loss. This impact will not be fully realised until the project is completed.

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

New knowledge gained will be disseminated through presentation at suitable conferences to researchers working in this field, veterinarians and horse caregivers and publication in suitable journals and lay articles. This will include publication of unsuccessful approaches.

Species and numbers of animals expected to be used

- Ponies: 25
- Horses: 25

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.

It is not possible to achieve the objectives of this project without using animals, as we are studying complex metabolic pathways and physiological responses, that cannot with our current state of knowledge, be modeled using isolated tissues, cells or computer simulations.

Laminitis is a disease which affects adult horses and ponies and it is therefore most appropriate to undertake these studies in these animals. Whilst we can model some aspects of digital vascular physiology and fat, muscle and caecal function *in vitro, t*he unique metabolism of the horse is central to the pathophysiology of the endocrinopathic laminitis. Thus, our studies to further elucidate the role of adiponectin in the pathogenesis of the disease require *in vivo* experiments.

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

Typically, animals will undergo each of the procedures a maximum of twice in this project.

Protocol 1 involves placement of intravenous catheters in each jugular vein under local anaesthesia and infusion of glucose and infusion via one catheter and collection of blood samples via the second catheter. It also involves a single intramuscular injection of corticosteroid followed by blood sample collection via jugular venepuncture.

Protocol 2 involves consuming sufficient pasture to promote natural weight gain. Blood samples will be obtained weekly by jugular venepuncture until each animal becomes overweight. This will be followed by weight loss achieved through consuming a hay-based diet with or without supplementation using nutritional supplements or pharmacologic agents administered orally. Blood samples will be obtained weekly by jugular venepuncture until each animal reaches ideal weight.

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

Studies conducted under this licence should not induce long term adverse effects in the 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)?

All protocols will be mild for all animals.

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

- Kept alive

- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 16 June 2026

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?

It is not possible to achieve the objectives of this project without using animals as we are studying complex metabolic pathways and physiological responses, including some that are influenced by season, that cannot with our current state of knowledge, be modelled using isolated tissues, cells or computer simulations.

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

Isolated equine tissues or cells and computer simulations were considered for use in this project. *In vitro* studies will inform which drugs to use in protocol 3 and help understanding of the mechanisms involved in adiponectin release from cultured adipocytes. The combination of *in vitro* and *in vivo* approaches is more powerful scientifically than either on its own.

Why were they not suitable?

Whilst *in vitro* studies will be used to inform the choice of pharmacologic agents used in protocol 3, they are not suitable as they do not take into account the complex physiological responses and complex metabolic pathways that all interact in the pathogenesis of endocrinopathic laminitis.

A retrospective assessment of replacement will be due by 16 June 2026

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?

The number of animals to be included in this project has been based on sample size calculations and on the assumption that animals will be re-used between protocols in order to reduce the overall number of animals used. Data from our previous studies involving measurement of circulating adiponectin concentrations in healthy ponies as well as weight gain and weight loss studies have been used to inform these studies. In each case, group sizes of six animals are sufficient. In addition, in previous *in vivo* equine studies, we have found that group sizes of six animals have been sufficient to produce robust results. Protocol 3 requires three groups of animals equating to a total of 18 animals. Thus, it is estimated that 25 animals will be used in total to allow for illness, accidental injury and the need to repeat a study in an individual animal for experimental reasons.

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

The number of animals to be included in this project has been based on sample size calculations and on the assumption that animals will be re-used between protocols in order to reduce the overall number of animals used.

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

The numbers to be used in this project are based on sample size calculations and previous experience.

A retrospective assessment of reduction will be due by 16 June 2026

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.

Protocol 1

The methods used to induce hyperinsulinaemia (high blood insulin concentrations) and tissue insulin resistance have been previously validated for ponies. The duration of the hyperinsulinaemia chosen is much shorter than that which has been previously reported to induce laminitis in healthy ponies. In addition, there is no scientific evidence to link corticosteroid administration with the development of laminitis in healthy animals, only in those with other laminitis risk factors. thus, only healthy, ideal weight animals with no history of previous laminitis will be used. Jugular catheters will be placed using local anaesthetic in order to minimise any pain and distress potentially associated with repeated blood sampling.

Protocol 2 and 3

The pasture-induced weight gain will be undertaken in consultation with an equine nutrition specialist to ensure that there is a gradual gain in weight over 9-12 weeks. Previous studies using this approach have not resulted in any adverse effects. The weight loss will be induced by feeding a diet low in non structural carbohydrate (<10% dry matter) at 1.25% (dry matter intake; DMI) of body weight. This is standard dietary change that is recommended by veterinary surgeons for weight loss in clinical cases of equine obesity when owners have allowed their animals to become obese. Thus, this will mimic somethings that happens in the real world. In those animals with weight loss resistance (animals that fail to lose weight despite dietary restriction), the diet may need to be reduced to 1% (DMI) of body weight, but no lower as lower percentages are associated with gastrointestinal disturbances such as gastric ulcers and the development of stereotypies (repetitive abnormal behaviours). The methods that will be used to assess the effect of the weight gain and subsequent weight loss on insulin metabolism (i.e. oral sugar test and insulin tolerance test) are methods that are commonly used in equine clinical practice.

Thus, all of these methods proposed have been previously validated for use in ponies in either the clinical or the research setting. Animals will be returned to their normal management regime between protocols which will involved continuous access to pasture. All of the protocols are mild in severity and none of the methods will cause lasting harm to the animals.

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

As previously explained, all of the experiments proposed need to be performed in horses and ponies. As equine metabolism differs significantly between the foetus, neonate and adult, and laminitis is a disease that only affects adult horses and ponies, we are unable to use a less sentient stage of life.

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

All animals will be habituated over several days to the room and the stocks in which the insulin infusion will be undertaken prior to commencement of the protocol.

All animals will be constantly monitored for the duration of all protocols and for the next 3 days to ensure that no adverse effects develop. If any adverse effects are noted, appropriate treatment will be provided immediately in consultation with the named veterinary surgeon.

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

We will follow the NC3Rs advice on the 3Rs for project licence holders.

The NC3Rs General Principles for blood sampling applicable to horses will be followed as well as the recommendations relating to use of vascular catheters.

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

We will stay informed of advances in 3Rs via the 3Rs Liaison, as well as via the NC3Rs website and attending relevant seminars/talks. Wherever we find an opportunity to improve our technique/experimental design to minimise animal numbers and/or suffering, we will rapidly incorporate it into our protocols. We will closely work to ensure that our animal care is always optimal, which ultimately ensure high quality results.

A retrospective assessment of refinement will be due by 16 June 2026

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?