G: NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed project clearly using non-technical terms which will be understandable to a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary may render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at http://scienceandresearch.homeoffice.gov.uk/animal-research/).

(WORD LIMIT: 1000 WORDS)

Please complete the following:

Project Title (max. 50 characters)	Angiogenesis in health and disease	
Key Words (max. 5 words)	Capillaries, blood flow, skeletal muscle, ischaemia, hypoxia	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals[1]

[1] At least one additional purpose must be selected with this option.

The Home Office, in line with the rest of HMG, has implemented the Government Security Classification (GSC). Details of the GSC can be found at https://www.gov.uk/government/publications/government-security-classifications. Please note that documents and emails you receive may contain specific handling instructions.

Handling Instructions: Contains personal sensitive information, subject to confidentiality requirements under the Data Protection Act. This should only be circulated in accordance with ASPA Guidance and stored in a locked secure location.

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Adequate control over the extent of the microcirculation is critical in health and disease, with numerous pathologies characterised by either uncontrolled expansion or failure to elicit adequate growth. We have identified a number of new avenues that require further work to mature the topic, mainly based around the ability of the cells making up the capillaries (or nearby perivascular cells) to sense changes in the local mechanic environment. We aim to exploit these models further in two ways: a) better understand the cellular and molecular regulation involved and b) refine the animal models of exercise to provide a more targetted intervention.
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)?	We may be able to avoid 'off-target' effects of many current pharmacology-based angiotherapies, optimise the efficacy of other angiotherapies, and provide guidance about specific forms of exercise that would most benefit individual patient groups.
What species and approximate numbers of animals do you expect to use over what period of time?	In the main we will use rats due to the appropriate size for invasive surgery and challenging terminal experiments (~1500/5 years), with mice used where genetic modification or expensive molecular interventions are required (~500/5 years), and rabbits where we wish to more closely mirror surgical interventions conducted in patients (~150/5 years).
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects are likely to be mainly in response to surgery, as identified in each of the protocols. From past experience of animal welfare monitoring the maximum level of severity will be moderate. The animals will be humanely killed after experimentation.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Alternatives to animals cannot be used to meet the aims and objectives of this work because *in vitro* models of angiogenesis differ fundamentally from the *in vivo* process. Many of the characteristics of endothelial and smooth muscle cells are altered in tissue culture systems, the contextual significance of extracellular matrix molecules and interstitial cell types is lacking, mechanical stimuli are usually absent, and the time course of adaptations cannot be mimicked realistically. The different factors initiating or controlling angiogenesis *in vivo* have also been proved to be different from those *in vitro*. The integrative nature of *in situ* tissue function is thus of paramount importance to the problems being addressed and the scientific rationale of the work is best served by use of animal models. Although it is encouraging that such data may be translated into the clinical setting, fundamental research is still needed in order to understand the mechanisms involved. Such investigations are impossible without the use of animals, all alternatives having been shown to be of limited value. Where parallel studies have been made (e.g. muscle ischaemia, hypoxia/COPD, elevated shear stress), qualitatively similar processes have been observed in animals and humans, giving confidence to continue to develop the animals models to inform clinical interventions. This work has progressed slowly in part because there are very few, specific inhibitors that can be used to probe the role of these signals *in vivo*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Appropriate experimental design and data analysis are routinely employed to minimise sample size without compromising the validity of outcomes. Sample sizes may be set using statistical insight ('power analysis', where the least practical difference between groups and the likely variability of the data is estimated to determine how many animals/group are required). Exact numbers of animals required will vary depending on the nature of the experimental intervention; in practice group sizes of 6-10 have been required in past experiments, with typically a 1-2% failure rate. Given genetic drift in commercial stocks, it has not been possible to reduce animal numbers by repeat analysis of control groups, though we continue to try by means of loop experimental design.

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Experiments will be carried out mainly using rodents as appropriate mammalian models to develop fundamental knowledge that may act as a prelude to veterinary application and human intervention. Previous experience has allowed refinement of the procedures in order to obtain reproducible angiogenesis in a relatively short duration, with reduced trauma. This facilitates the study and interpretation of mechanisms in a minimum number of animals. Mice will be one target because the use of transgenic and knockout technology can be used to investigate further the molecular basis of observations made in the rat. Sometimes the use of genetically-altered animals may be the only way to establish the *functional* role of a e.g. protein in supporting angiogenesis. In general, we aim to minimise harm by reducing: the frequency and/or duration of the procedure, the likelihood of known adverse effects and the proportion of animals likely to be affected, and the severity level by range-sighting to optimise efficiency. In addition, we adopt a monitoring regime whereby animals are closely monitored following invasive surgery, assisted by approved welfare assessment protocols, and subject to humane end-points. Triggers for interventions are stipulated in each protocol SOP. Genetically-altered animals may have an increase in susceptibility to infection, although this risk is considered to be minor in consideration of the high standards of husbandry in the CEU and health status of the mice. They may have increase in susceptibility to haemorrhage due to weak blood vessel structure, but this is considered unlikely to be a problem because mice are unlikely to experience excessive trauma in the BMSU, and there exists compensatory pathways in vessel maturity that would help to prevent an excessive loss of blood. A register will be maintained all genotypes/phenotypes and associated adverse effects, to be available for inspection by all personal licencees. Adverse effects that have been noted prior to transfer of the animals from another project licence will also be recorded on the register. Any newly identified adverse effects in wildtype and genetically modified animals will be recorded along with the appropriate route of action to be taken. The register will guide control measures and humane endpoints, and will be used to record the phenotype of new strains of mice for which we do not have specific information.

For Office Use Only				
Will the project be subject to Retrospective Assessment?[1]	Yes	No	Date due:	

Appendix 1

Protocol 1: worse case = pregnant animal with implanted stimulator, muscles stimulated, VO2 measured on a treadmill, with infusion of markers under terminal anaesthesia. Usual case = no first (pregnancy unlikely to be studied as a main physiological challenge, dietary restriction usually prior to surgery to minimise trauma) or second optional interventions (these help refine basic findings where needed).

Protocol 2: worse case = pregnant animal with implanted slow release device having VO2 measured on a treadmill, with infusion of markers under terminal anaesthesia. Usual case = no first (pregnancy unlikely to be studied as a main physiological challenge, dietary restriction usually prior to surgery to minimise trauma) or second optional interventions (these help refine basic findings where needed).

Protocol 3: worse case = pregnant animal with unilateral muscle extirpation having VO2 measured on a treadmill, with infusion of markers under terminal anaesthesia. Usual case = no first (pregnancy unlikely to be studied as a main physiological challenge, dietary restriction usually prior to surgery to minimise trauma) or second optional interventions (these help refine basic findings where needed).

Protocol 4: worse case = pregnant animal with muscle implants, unilateral arterial ligation and drug-eluting stent having VO2 measured on a treadmill, and infusion of markers under terminal anaesthesia. Usual case = no first (pregnancy unlikely to be studied as a main physiological challenge, dietary restriction usually prior to surgery to minimise trauma) or second optional interventions (these help refine basic findings where needed).).

Protocol 5: worse case = pregnant animal subjected to progressive hypoxia having VO2 measured on a treadmill, with infusion of markers under terminal anaesthesia. Usual case = no first (pregnancy unlikely to be studied as a main physiological challenge, dietary restriction usually prior to surgery to minimise trauma) or second optional interventions (these help refine basic findings where needed).

Protocol 6: worse case = pregnant animal with unilateral muscle extirpation subjected to indirect electrical stimulation *via* implanted electrodes having VO2 measured on a treadmill, with infusion of markers under terminal anaesthesia. Usual case = no first (pregnancy unlikely to be studied as a main physiological challenge, dietary restriction usually prior to surgery to minimise trauma) or second optional interventions (these help refine basic findings where needed).