East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

July 3, 2012

David Tulis, Ph.D.
Department of Physiology
Brody 6N-98
ECU Brody School of Medicine

Dear Dr. Tulis:

Your Animal Use Protocol entitled, "AMP Kinase Control of Mouse Vascular Smooth Muscle Growth" (AUP #Q312) was reviewed by this institution's Animal Care and Use Committee on 7/3/12. The following action was taken by the Committee:

"Approved as submitted"

Please contact Dale Aycock at 744-2997 prior to hazard use

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

enclosure

East Carolina University is a constituent institution of the University of North Carolina. An equal opportunity university.

East Carolina University
Animal Use Protocol (AUP) Form
Latest Revision, July, 2010

Project Title: AMP Kinase control of mouse vascular smooth muscle growth

1. Personnel

1.1. Principal investigator and email: David A. Tulis, Ph.D.; tulisd@ecu.edu

1.2. Department, office phone: Physiology; 252-744-2771

1.3. Emergency numbers:

Name:	David A. Tulis, Ph.D.	Joshua D. Stone
Cell:	919-491-2906	828-231-8222
Pager:		
Home:	252-353-5957	

FOR IACUC USE ONLY

AUP # 12312
New/renewal: New
Date received: 6/27/12
Full Review and date: Designated Reviewer and date:
Approval date: 7/3/12
Study type: blood vessel diseases
Pain/Distress category: D
Surgery: ✓ Survival: ✓ Multiple:
Prolonged restraint:
Food/fluid restriction:
Hazard approval/dates: Rad: IBC: ✓ EH&S:
OHP enrollment/mandatory animal training completed :
Amendments approved:

6E-71, 6E-108 - arteriotomy

CA-AMPK

1.4. Co-Investigators if any: None.

1.5. List all personnel (PI, Co-I, technicians, students) that will be performing procedures on live animals and describe their qualifications and experience with these specific procedures. If people are to be trained, indicate by whom:

Name	Required ECU Training	Other Relevant Animal Experience/ Training
David A. Tulis, Ph.D., PI	Yes, completed.	~20 years experience in rat and mouse survival surgeries
Joshua Stone	Yes, completed.	~2 ½ years experience in rat and mouse surgeries; trained in surgical procedures by the PI.
Patti Shaver	Yes, completed.	~2 years experience in rodent surgeries; trained in surgical procedures by the PI.
Jackson Vuncannon	Yes, completed.	Will be trained in surgical procedures by the PI & Joshua Stone.

2. Regulatory Compliance

2.1 Non-Technical Summary

Using language a non-scientist would understand, please provide a 6 to 8 sentence summary explaining the overall study objectives and benefits of proposed research or teaching activity, and a brief overview of all procedures involving live animals (more detailed procedures are requested later in the AUP). Do **not** cut and paste the grant abstract.

The overall objective of this project is to study blood vessel diseases with the hope of finding out what their causes are and discovering cures for them. We will study the effects of a known signaling pathway, AMP kinase, which may be protective against abnormal growth in blood vessels. We will breed and use mice that are deficient in this factor. With experimental mice, we will perform carotid artery surgery similar to surgeries performed in humans who have blood vessel diseases. Animals must be used for these experiments because we need to obtain tissues after surgery in order to measure changes in certain factors that may be involved in disease processes. These changes are only "real" in a whole animal setting and cannot be observed through non-animal (ie, isolated tissue; computer simulation) approaches. Moreover, the use of mice allows for genetic manipulation of specific factors that is not currently available in other experimental animal models. This is the case in this project through the use of genetically-altered AMP kinase-deficient mouse models. Throughout these studies, every effort will be made to ensure that the animals are comfortable.

2.2. Duplication

Does this study duplicate existing research? Yes ☐ No ☒

If yes, why is it necessary? (note: teaching by definition is duplicative)

2.3 Alternatives to the Use of Live Animals

Are there less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation that can be used in place of the live vertebrate species proposed here? Yes ☐ No ☒

If yes, please explain why you cannot use these alternatives.

2.4 Literature Search to ensure that there are no alternatives to all potentially painful and/or distressful procedures

List the following information for each search (please do not submit search results but retain them for your records):

Date Search was performed: June 06, 2012
Database searched: NIH PubMed/Medline; NIH Reporter
Period of years covered in the search: All (specific years were not defined).
Keywords used and strategy: mouse; mice; vascular remodeling; neointima; alternative; wire injury; denudation; carotid artery; AMP kinase; AMPK; adenosine monophosphate protein kinase

Other sources consulted:

Narrative indicating the results of the search (2-3 sentences) and explaining why there are no alternatives to your proposed procedures that have the potential to cause pain and/or distress. If alternatives exist, describe why they are not adequate.

The PI performed extensive literature and database searches for acceptable alternatives to animal models for similar vascular surgeries. No adequate alternatives were found that would be relevant to the proposed studies described herein. Alternate animal models or viable alternatives to animal models (computer models, etc.) that accurately replicate the human arterial response to injury are not currently available; however, the PI will continue to search for acceptable alternatives for animal models on a regular basis in the future through PubMed/Medline and the NIH Reporter. Every effort will be made by the PI to reduce, refine, and replace animal models with non-animal approaches whenever possible. Specifically, we will keep the animal numbers at a minimum for what is scientifically required to achieve sound results. We will refine our techniques wherever possible in order to minimize pain and discomfort, and if non-animal models are found in the future we will replace our current models with those.

2.5 Hazardous agents

2.5a. Protocol related hazards

Please indicate if any of the following are used in animals and the status of review/approval by the referenced committees:

HAZARDS	Oversight committee	Status (Approved, Pending, Submitted)/Date	AUP Appendix 1 Completed?
Radioisotopes	Radiation		
Ionizing radiation	Radiation		
Infectious agents (bacteria, viruses, rickettsia, prions)	IBC		
Toxins of biological origins (venoms, plant toxins, etc.)	IBC		
Transgenic, Knock In, Knock Out Animals---breeding, cross breeding or any use of live animals or tissues	IBC	Submitted (06/2012) 6/29/12	
Human tissues, cells, body fluids, cell lines	IBC		
Viral/ Plasmid Vectors/ Recombinant DNA or recombinant techniques	IBC	Submitted (06/2012) 6/29/12	06/2012
Oncogenic/toxic/mutagenic chemical agents	EH&S		
Nanoparticles	EH&S		
Cell lines injected or implanted in animals (MAP test)	DCM		
Other agents:	IBC and EH&S		

2.5b. Incidental hazards

Will personnel be exposed to any incidental zoonotic diseases or hazards during the study (field studies, primate work, etc)? If so, please identify each and explain steps taken to mitigate risk:

No.

3. Animals and Housing

3.1. Species and strains:

Species: *Mus musculus*
Strains:
Mice: C57BL/6J background; WT mice; homozygous "flox" AMPK alpha1 and alpha2 mutant mice (strain name: STOCK *Prkaa1*$^{tm1.1Sjm}$/J; stock number 014141, and B6(Cg)-*Prkaa2*$^{tm1.1Sjm}$/J; stock number 014142 respectively); hemizygous smooth muscle-specific Cre-recombinase-expressing mice (driven by *Myh11*, a smooth muscle-specific promoter and containing a traceable GFP tag; strain name B6.Cg-Tg(Myh11-cre,-EGFP)2Mik/J; stock number 007742) (purchased from The Jackson Laboratory; www.jax.org).

3.2. Weight, sex and/or age:

18-26 grams; male and female (breeding pairs)

Total number of animals in treatment and control groups	Additional animals (Breeders, substitute animals)	Total number of animals used for this project
192	704	896

* See attached for group numbers per animal goal

3.3. Justify the species and number (use statistical justification when applicable) of animals requested:

Overview of surgical procedure: The overall goal of this project is to evaluate the influence of AMPK on vascular remodeling. AMPK has two isoforms (alpha1 and alpha2); therefore, mice with each isoform deleted will be used to test the AMPK response to vascular injury compared to WT mice. To assess this we will perform surgery on the mice denuding the left carotid artery, which will then be followed up after 4 weeks with euthanasia and tissue harvesting. Therefore, since 12 mice are needed per cohort in this whole animal approach to achieve required significance **896 mice** will be used over the 2 years of this project (this number includes those mice which will be used for breeding and for tissue harvest). The mouse model of carotid artery injury is an acceptable animal model for replicating the human response to vessel injury that has been extensively used for many years and that is established in our laboratory. The PI currently has an approved ECU AUP using this model (AUP #Q261). Several seminal references along with the original description of the mouse denudation injury (Ref. #5) and two recent book chapters (written by Dr. Tulis) that detail these whole animal procedures and that also provide rationale for use of rodents in these studies are:

1. Clowes, A.W., Reidy, M.A., and Clowes, M.M. Mechanisms of stenosis after arterial injury. *Lab. Invest.* 49, 208-215, 1983.
2. Clowes, A.W., Reidy, M.A., and Clowes, M.M. Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium. *Lab. Invest.* 49, 327-333, 1983.

3. Lindner, V., Fingerle, J., Reidy, M.A. Mouse model of arterial injury. Circ. Res. 73: 792-796, 1993.
6. Tulis, D.A. Rat Carotid Artery Balloon Injury Model, *Methods Mol. Med.* 139: 1-30, 2007.
7. Tulis, D.A. Histological and Morphometric Analyses for Rat Carotid Artery Balloon Injury Studies, *Methods Mol. Med.* 139: 31-66, 2007.

Overview of breeding: For breeding, knock-in lines carrying a "flox" (flanked by *loxP*) AMPK alpha1 or alpha2 allele (strain name: STOCK *Prkaa1*$^{tm1.1Sjm}$/J; stock number 014141, and B6(Cg)-*Prkaa2*$^{tm1.1Sjm}$/J; stock number 014142 respectively) or a *Cre* recombinase allele (driven by *Myh11*, a smooth muscle-specific promoter and containing a traceable GFP tag; strain name B6.Cg-Tg(Myh11-cre,-EGFP)2Mik/J; stock number 007742) on a C57BL6 background will be used. Crossing these lines will generate offspring that have exon 3 of AMPK alpha1 or exon 2 of AMPK alpha2 deleted only in the Cre-expressing (driven by Myh11) smooth muscle. Mice that are AMPK alpha1$^{-/-}$ or alpha2$^{-/-}$ are viable, fertile, normal in size and show no abnormal phenotype or gross physical or behavioral abnormalities. Additionally, use of smooth muscle-specific AMPK alpha1 and alpha2 KO mice has been recently described (Circulation Research 109; 1230-1239, 2011).

Estimation of animal numbers: For estimation of the numbers of animals needed per treatment group (variable per endpoint analyzed; see full description below) for the mouse studies described in this protocol, previous experience with these surgeries and associated morbidity/mortality data was used and combined with the statistical analysis programs SigmaPlot 11.0 for Windows and Excel 2007 for consultation. The PI input data from previous experiments, including approximate sample size, mean, and standard error of the mean. Duplicity of use for specific tissues (discussed below) was considered. For these inclusive experiments, statistical power remained between 0.85 and 1.00, indicating that the sensitivity of these experiments is high and should detect any true differences in the data if differences truly exist. These experiments were designed to achieve the most data from each animal, and when possible, numerous tissues are obtained (i.e., both control(s) and treatment) from each animal to keep the total numbers of animals at a minimum. A single batch of similarly processed tissues, paraffin-embedded tissues for example, will be used for redundant analyses (i.e., morphometry, immunostaining for AMPK). This avoids un-necessary duplication of animal experimentation and animal numbers. For proper scientific purposes, however, appropriate control animal groups (including WT and AMPK KO mice prior to Cre-recombination) will be used for comparison. A single control group may be used in more than one experiment, provided that it represents an appropriate control for comparison to any specific experiment.

This project has two components: an experimental component consisting of cell culture and remodeling studies and a breeding component. Based on a 2 year duration for this protocol and considering both experimental (remodeling and cell culture) and breeding purposes, a **total of 896 mice will be needed.** For the remodeling experiments, paraffin-embedded tissues used for the 4-week histological analysis will also be used at the 4-week time point for verification (via immunostaining) of AMPK alpha1 and alpha2 ablation (along with analysis of

potential induction of reciprocal alpha subunit). Cohorts for the remodeling studies will need 12 animals each and will include the following: (1) WT mice; (2) AMPK alpha1-deficient mice; (3) AMPK alpha2-deficient mice; (4) WT mice with local delivery of constitutively active AMPK (CA-AMPK); (5) AMPK alpha1-deficient mice with local delivery CA-AMPK; and (6) AMPK alpha2-deficient mice with local delivery CA-AMPK. Thus, we will need 24 WT mice, 24 AMPK alpha1 KO mice, and 24 AMPK alpha2 KO mice for a total of 72 animals. For cell culture, 20 animals per year will be needed for each cohort: WT, AMPK alpha 1-deficient, and AMPK alpha 2-deficient mice, **thus a total of 120 animals will be required for the cell culture portion of this project. Therefore, a total of 192 animals (72 for remodeling studies, 120 for cell culture studies) will be needed for experimental purposes of this project.**

Estimates for animal numbers for breeding were constructed in consultation with Dr. Chris Geyer, Assistant Professor, Department of Anatomy and Cell Biology and Collaborator on this project. Breeding of alpha1$^{flox/+}$;SM-Cre and alpha2$^{flox/+}$;SM-Cre mice with AMPK alpha1$^{flox/flox}$ or alpha2$^{flox/flox}$ mice will yield 25% offspring with a deletion of AMPK alpha specifically in smooth muscle. Throughout this entire breeding process, all mice will be genotyped by PCR using DNA isolated from tail tips.

To begin this project, two alpha1$^{flox/flox}$, two alpha2$^{flox/flox}$, and two SM-Cre breeding pairs will be purchased from The Jackson Laboratory for a total of 12 mice (6 breeding pairs). Since these mice are on a C57Bl/6 background, we will conservatively estimate 8 pups per litter with about 1 litter per month over a reproductive timeframe of 5 months for approximately 40 animals per breeding pair. Three different breeding schemes will be followed:

1. To continuously maintain the production of alpha1$^{flox/flox}$ and alpha2$^{flox/flox}$ mice, we will set up one of the breeding pairs from The Jackson Laboratory for each floxed gene (40 for alpha1 and 40 for alpha2).
2. The other breeding pair will be bred with SM-Cre mice (again, 40 for alpha1 and 40 for alpha2) in order to generate alpha1$^{flox/+}$;SM-Cre and alpha2$^{flox/+}$;SM-Cre mice, respectively. Thus, in order to generate alpha1$^{flox/flox}$ and alpha2$^{flox/flox}$ mice and alpha1$^{flox/+}$;SM-Cre and alpha2$^{flox/+}$;SM-Cre mice, **we anticipate needing a total of 160 mice for steps 1 and 2.**
3. Next, in order to generate SM tissue-specific KO mice, we will breed alpha1$^{flox/+}$;SM-Cre and alpha2$^{flox/+}$;SM-Cre mice with alpha1$^{flox/flox}$ and alpha2$^{flox/flox}$ mice, respectively. Assuming 8 pups/litter with 1 litter/month over 5 months, we will need 240 animals to generate alpha1 KO and 240 animals to generate alpha2 KO (**total estimate of 480animals for step 3). Totally, this yields a total of 640 mice needed for breeding.**

Additionally, adding an extra 10% (64) to account for unforeseen complications in generating the required phenotype, **we anticipate a total of 704 animals will be needed for breeding purposes. Combining animal numbers for the experimental studies (192)**

and breeding purposes (704), we estimate that we will use **a total of 896 mice for both years of this protocol.** The table below summarizes this information, and a schematic is included (next few pages) diagraming this breeding scenario:

Experiment:	# Mice
4 week remodeling studies and cell culture	192
+ Breeding total	704
= GRAND TOTAL FOR 2 YEARS:	896

For data interpretation and statistical analyses of all results, since both control and treatment arterial sections will be obtained from the same animal, a paired Student's t-test will be performed. For inter-animal comparisons between treatment groups, an ANOVA (with appropriate multiple comparisons post-hoc tests) and/or unpaired t-tests will be used. Unless otherwise specified, all data will be represented as mean ± standard error of the mean. A significance level (p-value) less than 0.05 will be used for all comparisons.

3.4. Justify the number and use of any additional animals needed for this study (i.e. breeder animals, inappropriate genotype/phenotype, extra animals due to problems that may arise, etc.):

For breeding purposes and potential loss due to complications in generating the required phenotype, 10% of the estimated breeding total of 640 (which equals 64 additional animals) is added into the numbers described in Section 3.3 above.

Health & Colony Maintenance Information

Animal Health Reports

Room Number AX11

Colony Maintenance

Breeding & Husbandry When maintaining a live colony, hemizygous smMHC/Cre/EGFP mice can be bred to wild-type siblings or C57BL/6J inbred mice. Homozygotes are viable and fertile, with smaller litter sizes and a higher incidence of perinatal mortality. Because transgene expression is observed in both maternal and paternal germlines (see Strain Phenotype description for details), it is recommended to maintain the smMHC/Cre/EGFP transgenic colony without additional "floxed" mutations in their genome.

Mating System Noncarrier x Hemizygote (Female x Male) 02-JUL-08

Diet Information

3.5. Will the phenotype of mutant, transgenic or knockout animals predispose them to any health behavioral, or physical abnormalities? Yes ☐ No ☒ (if yes, describe)

3.6. Are there any unusual husbandry and environmental conditions required? Yes ☐ No ☒ If yes, then describe conditions and justify the exceptions to standard housing (temperature, light cycles, sterile cages, special feed, feed on cage floor, prolonged weaning times, wire-bottom cages, no enrichment, social isolation, etc.):

3.7. If wild animals will be captured or used, provide permissions (collection permit # or other required information):

Not applicable.

3.8. List all laboratories or locations outside the animal facility where animals will be used. Note that animals may not stay in areas outside the animal facilities for more than 12 hours without prior IACUC approval. For field studies, list location of work/study site.

All surgeries will be performed in the laboratory of the PI (in the Department of Physiology, Brody building 6th floor, rooms 6E-71 and 6E-108).

4. Animal Procedures

4.1. Will procedures other than euthanasia and tissue collection be performed? Yes ☒ No ☐

If animals will be used exclusively for tissue collection following euthanasia (answer "no" above), then skip to Question 5 (Euthanasia).

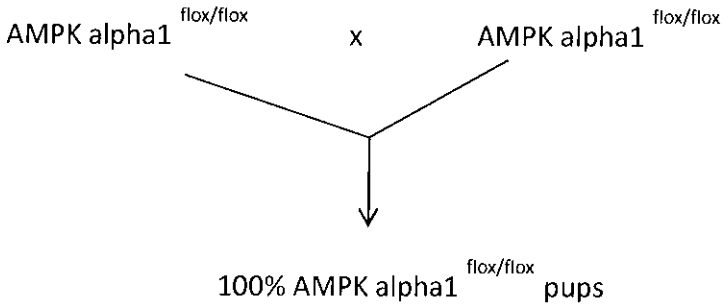
4.2. Outline the Experimental Design including all treatment and control groups and the number of animals in each. If this is a breeding protocol, please describe the breeding strategy (pairs, trios, etc.) and method and age of genotyping (if applicable). Tables or flow charts are particularly useful to communicate your design.

Methodologies for these studies involve carotid artery wire denudation injury on WT and smooth muscle-specific conditional AMPK alpha1 and alpha2 KO mice. Details of this protocol have been previously described (Lindner, V., Fingerle, J., Reidy, M.A. Mouse model of arterial injury. Circ. Res. 73: 792-796, 1993) and this procedure is fully established and operational in the laboratory of the mentor. Also, this protocol is currently approved by the ECU ACUC (protocol #Q261).

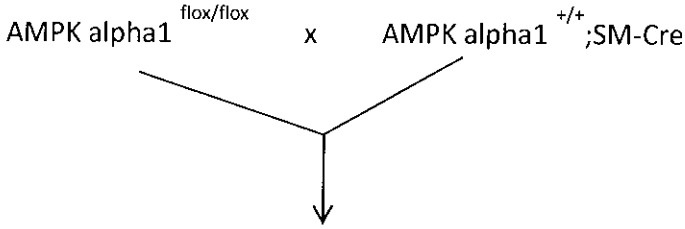
For remodeling experiments: These studies will use a total of 72 mice (n = 12 per cohort), and cohorts will include (1) WT mice; (2) AMPK alpha1-deficient mice; (3) AMPK alpha2-deficient mice; (4) WT mice with local delivery of constitutively active AMPK (CA-AMPK); (5) AMPK alpha1-deficient mice with local delivery CA-AMPK; and (6) AMPK alpha2-deficient mice with local delivery CA-AMPK. The surgical procedure that will be performed on mice will be wire denudation (removal of the endothelium) of the left common carotid artery. Regarding the surgery, first the animal will be anesthetized and will be provided analgesia, and then the neck area will be shaved of hair, and this area will be swabbed three times with surgical iodine and 70% alcohol prior to surgery. The animal will be laid supine on a heated operating table, and legs and head will be retracted carefully. The skin will be opened with a midline incision along the ventral aspect of the neck. Underlying tissues will be blunt dissected to expose the common carotid and external carotid artery branch. Again using blunt dissection, the area immediately surrounding the bifurcation will be cleared, and a surgical micro-clamp will be placed on the common carotid for hemostasis. A small arteriotomy will be made on the external carotid branch, and an embolectomy catheter guide wire will be inserted into the common carotid and advanced (with removal of the clamp) and withdrawn thrice. The wire will be removed and in cohorts (4) - (6) a replication deficient adenovirus for constitutively active AMPK (CA-AMPK) will be infused luminally (15 uL) for 30 minutes, the virus will be removed and the lumen flushed with saline and a suture will be tied around the arteriotomy incision on the external branch. Vessel patency and pulsatility will be checked immediately following surgery, and the overlying tissues will be sutured and the skin closed using standard small rodent skin clips. The area surrounding the incision will be swabbed with an antiseptic/antimicrobial agent, the animal will be provided supplementary fluids and the animal will be kept on the heated surface under supervision until full recovery. The animal will then be returned to the housing room and provided food and water ad libitum. Animals will be maintained for 4 weeks and changes in vessel wall remodeling induced by AMPK alpha1 and/or alpha2 deficiency will be measured histologically (medial wall area, neointimal area, vessel perimeters). Throughout these procedures the animals will be monitored daily by lab personnel for the following signs of distress (which, if overtly evident, will lead to early euthanasia): immobility, huddled (hunched) posture, inability to eat or drink, ruffled fur, self-mutilation, vocalization, wound dehiscence, hypothermia, or greater than 20% weight loss. The animals will then be sacrificed, and the appropriate tissues obtained for subsequent analyses.

Tissue harvesting: For obtaining primary cells for culture, 120 total mice will be needed. This will maintain a sufficient working stock of cells to be used for a variety of cell culture studies. For IACUC purposes, these studies involve anesthetic overdose of animals (2-2.5x surgical dose) followed by exsanguinations and pneumothorax. After death is assured, tissues will be removed as needed.

Step 1: to maintain production of alpha1$^{flox/flox}$ mice (and alpha2$^{flox/flox}$ mice)

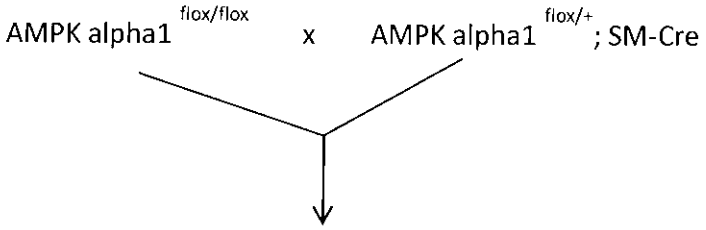


Step 2: to generate alpha1$^{flox/+}$; SM-Cre mice



50% AMPK alpha1$^{flox/+}$; SM-Cre (save; breed with AMPK alpha1$^{flox/flox}$ mice)
50% AMPK alpha1$^{flox/+}$; WT (discard or use as controls)

Step 3: to generate SM-specific alpha1 KO mice



25% AMPK alpha1$^{flox/flox}$; SM-Cre (SM-specific KO)

25% AMPK alpha1$^{flox/flox}$; WT
25% AMPK alpha1$^{flox/+}$; SM-Cre (het controls)
25% AMPK alpha1$^{flox/+}$; WT

In sections 4.3-4.19 below, please respond to all items relating to your proposed animal procedures. If a section does not apply to your experimental plans, please leave it blank.

Note: Procedures covered by DCM and IACUC guidelines and policies are indicated by asterisk (*). Please refer to these and justify any departures.

4.3. Anesthesia/Analgesia/Tranquilization/Pain/Distress Management (for procedures other than surgery)

Adequate records describing anesthetic monitoring and recovery must be maintained for all species.

If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary.

Not applicable.

Describe the pre-procedural preparation of the animals:

1a. Food restricted for _____ hours

1b. Food restriction is not recommended for rodents and rabbits and must be justified:

2a. Water restricted for _____ hours

2b. Water restriction is not recommended in any species for routine pre-op prep and must be justified:

	Agent	Concentration	Dose (mg/kg)	Volume	Route	Frequency	Duration
Pre-emptive analgesic	Meloxicam	5 mg/mL	5-10 mg/kg BW	25 uL	PO	Once prior to surgery; 1/24 hours afterwards as needed.	Recommend day of and day after surgery, then as needed
Pre-anesthetic							
Anesthetic	DCM cocktail for	ketamine (90 mg/ml),	90 mg/kg	0.1 ml / 10 g BW	IP	1 (prior to surgery);	Throughout the

	mice	xylazine (10 mg/ml)	and 10mg/kg			supplemental doses (10% original dose) as needed	operative procedure in order to ensure adequate anesthesia
Other	Lidocaine		4 mg/kg BW	~50 ul	topical	2x during surgery	

4. Reason for administering agent(s):

b. For which procedure(s):

c. Method of monitoring anesthetic depth:

d. Methods of physiologic support during anesthesia and recovery:

e. Duration of recovery:

f. Frequency of recovery monitoring:

g. Specifically what will be monitored?

h. When will animals be returned to their home environment?

4.[illegible]. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort:

4.4 Use of Paralytics

Will paralyzing drugs be used?

No.

For what purpose:

Please provide scientific justification for paralytic use:

Paralytic drug:

Dose:

Method of ensuring appropriate analgesia during paralysis:

4.5. Blood or Body Fluid Withdrawal/Tissue Collection/Injections/Tail Snip*/Gavage

Please fill out appropriate sections of the chart below:

	Location on animal	Needle/ catheter/ gavage tube size	Route of administration	Biopsy size	Volume collected	Compound and volume administered (include concentration and/or dose)	Frequency of procedure
Body Fluid Withdrawal							
Tissue Collection	Neck; thoracic cavity	N/A	N/A	Left and right carotid arteries	N/A	N/A	After euthanasia
Tail snip*	Tail	N/A	N/A	< 5 mm	N/A	N/A	Once, prior to weaning per ECU policy
Gavage							
Injection/Infusion	Carotid artery	N/A	luminal	N/A	N/A	CA-AMPK (15 uL; 30 minute incubation)	Intraoperatively

The only reagent that will be used in this study will be viral-mediated constitutively active AMPK (CA-AMPK), which will be performed as an "ad-back" intervention (in cohorts (4) - (6)) to restore AMPK activity in the injured carotid artery. This virus carrying CA-AMPK is a replication-deficient recombinant adenovirus (Diabetes (2005), 54, 1331-1339) that will not be shed so no special housing or procedures will be necessary. This reagent will be sterile and at physiologic pH (7.2 - 7.4) and will be stored frozen in sealed, air-tight Eppendorf cryo-tubes. Approaches using these agents have been reviewed by NIH and AHA study sections as well as by other IACUCs, and these experimental approaches have been peer-reviewed and previously published by this group [*J. Cardiovasc. Pharm. Ther.* 14 (2): 116-124, 2009; *Arterioscler. Thromb. Vasc. Biol.* 29 (4): 488-494, 2009; *American J. Therapeutics* 15: 551-564, 2008; *Methods Mol. Med.* 139: 1-30, 2007; *Methods Mol. Med.* 139: 31-66, 2007; *Arterioscler. Thromb. Vasc. Biol. 26: 85-90, 2006; Cell. Mol. Biol. 51:441-446, 2005. Diabetes. 2005;54(5):1331-9; The Journal of biological chemistry. 2003;278(31):28434-42.*]

4.6. Prolonged restraint with mechanical devices

Restraint in this context means ***beyond routine care and use procedures*** *for rodent and rabbit restrainers, and large animal stocks. Prolonged restraint also includes* ***any*** *use of slings, tethers, metabolic crates, inhalation chambers, primate chairs and radiation exposure restraint devices.*

a. For what procedure(s):

Not applicable.

b. Restraint device(s):

c. Duration of restraint:

d. Frequency of observations during restraint/person responsible

e. Frequency and total number of restraints:

f. Conditioning procedures:

g. Steps to assure comfort and well-being:

h. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.7 Tumor* and Disease Models/Toxicity Testing

a. Describe methodology:

Not applicable.

b. Expected model and/or clinical/pathological manifestations:

c. Signs of pain/discomfort:

d. Frequency of observations:

e. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.8 Treadmills/Swimming/Forced Exercise

a. Describe aversive stimulus (if used):

Not applicable.

b. Conditioning:

c. Safeguards to protect animal:

d. Duration:

e. Frequency:

f. Total number of sessions:

g. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.9 Projects Involving Food and Water Deprivation or Dietary Manipulation

(Routine pre-surgical fasting not relevant for this section)

a. Food Restriction

i. Amount restricted and rationale:

Not applicable.

ii. Duration (hours for short term/weeks or months for long term):

iii. Frequency of observation/parameters documented (weight, etc):

iv. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

b. Fluid Restriction

i. Amount restricted and rationale:

Not applicable.

ii. Duration (hours for short term/weeks or months for long term):

iii. Frequency of observation/parameters documented:

iv. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

c. Dietary Manipulations

i. Compound supplemented/deleted and amount:

Not applicable.

ii. Duration (hours for short term/weeks or months for long term):

iii. Frequency of observation/parameters documented:

iv. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.10 Endoscopy/Fluroscopy/X-Ray/Ultrasound/MRI/CT/PET/Other Imaging-complete for ultrasound/echo

a. Describe animal methodology:

b. Duration of procedure:

c. Frequency of observations during procedure:

d. Frequency/total number of procedures:

e. Method of transport to/from procedure area:

e. Please provide or attach appropriate permissions/procedures for animal use on human equipment:

4.11 Polyclonal Antibody Production*

a. Antigen/adjuvant used:

Not applicable.

b. Needle size:

c. Route of injection:

d. Site of injection:

e. Volume of injection:

f. Total number of injection sites:

g. Frequency and total number of boosts:

h. What will be done to minimize pain/distress:

i. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.12 Monoclonal Antibody Production

a. Describe methodology:

Not applicable.

b. Is pristane used: [ ] Yes [ ] No

- Volume of pristane:

c. Will ascites be generated: [ ] Yes [ ] No

d. Criteria/signs that will dictate ascites harvest:

e. Size of needle for taps:

f. Total number of taps:

g. How will animals be monitored/cared for following taps:

h. What will be done to minimize pain/distress:

j. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.13 Temperature/Light/Environmental Manipulations

a. Describe manipulation(s):

Not applicable.

b. Duration:

c. Intensity:

d. Frequency:

e. Frequency of observations/parameters documented:

f. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.14 Behavioral Studies

a. Describe methodology/test(s) used:

Not applicable.

b. If aversive stimulus used, frequency, intensity and duration:

c. Frequency of tests:

d. Length of time in test apparatus/test situation:

e. Frequency of observation/monitoring during test:

f. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.15 Capture with Mechanical Devices/Traps/Nets

a. Description of capture device/method:

Not applicable.

b. Maximum time animal will be in capture device:

c. Frequency of checking capture device:

d. Methods to ensure well-being of animals in capture device:

e. Methods to avoid non-target species capture:

f. Method of transport to laboratory/field station/processing site and duration of transport:

g. Methods to ensure animal well-being during transport:

h. Expected mortality rates:

i. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.16 Manipulation of Wild-Caught Animals in the Field or Laboratory

a. Parameters to be measured/collected:

Not applicable.

b. Approximate time required for data collection per animal:

c. Method of restraint for data collection:

d. Methods to ensure animal well-being during processing:

e. Disposition of animals post-processing:

f. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.17 Wildlife Telemetry/Other Marking Methods

a. Describe methodology (including description of device):

Not applicable.

b. Will telemetry device /tags/etc be removed? If so, describe:

c. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.18 Other Animal Manipulations

a. Describe methodology:

Not applicable.

b. Describe methods to ensure animal comfort and well-being:

c. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

4.19 Surgical Procedures

All survival surgical procedures must be done aseptically, regardless of species or location of surgery. Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained for all species.

A. Location of Surgery (Room #):

All surgeries will be performed in the laboratory of the PI (in the Department of Physiology, Brody building 6th floor, rooms 6E-71 and 6E-108).

B. Type of Surgery:

[ ] Nonsurvival surgery (animals euthanized without regaining consciousness)
[X] Major survival surgery (major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function)
[ ] Minor survival surgery

[ ] Multiple survival surgery*
If yes, provide scientific justification for multiple survival surgical procedures:

C. Describe the pre-op preparation of the animals:

1a. Food restricted for [0] hours

1b. Food restriction is not recommended for rodents and rabbits and must be justified:

[ ]

2a. Water restricted for [0] hours

2b. Water restriction is not recommended in any species for routine pre-op prep and must be justified:

[ ]

D. Minimal sterile techniques will include (check all that apply):

Please refer to DCM Guidelines for Aseptic Surgery for specific information on what is required for each species and type of surgery (survival vs. non-survival).

[X] Sterile instruments

- How will instruments be sterilized:

[Surgical instruments will be sterilized before use through autoclaving, and in between animals (between surgeries) using a glass bead sterilizer with incubation times of approximately 20 seconds (per manufacturers' instructions).]

If serial surgeries are done, how will instruments be sterilized between surgeries:

[For serial surgeries, instruments will be cleaned, washed in 79% ethanol, and then sterilized using a glass bead sterilizer as described above.]

[X] Sterile gloves

[X] Cap and mask

[X] Sterile gown

[X] Sanitized operating area

[X] Clipping or plucking of hair or feathers

[X] Skin preparation with a sterilant such as betadine

[X] Practices to maintain sterility of instruments during surgery

[X] Non-survival (clean gloves, clean instruments, etc.)

E. Describe all surgical procedures:

1. Skin incision size and site on the animal:

[The skin incision site will be on the ventral neck area, approximately 1 inch in length.]

2. Describe surgery in detail (include size of implant if applicable):

The skin will be opened along a midline incision on the ventral aspect of the neck approximately 1 inch long. Underlying tissue will be blunt-dissected to expose the common carotid and external carotid arteries. Blood flow through the common carotid will be temporarily stopped with an arterial clip, and an arteriotomy will be made on the external carotid branch. An embolectomy catheter guide wire will be inserted into the common carotid and advanced (with removal of the clamp) and withdrawn thrice. The wire will be removed and CA-AMPK (15uL) will be infused for 30 minutes. Following removal of the CA-AMPK the lumen will be flushed with warm saline and a suture will tied around the arteriotomy incision on the external branch. Vessel patency and pulsatility will be checked, the overlying tissues will be sutured using sterile 6-0 absorbable suture (Ethicon), and the skin closed using standard rodent skin clips. The animal will be provided supplementary fluids (0.9% normal saline, sterile, 1mL) via subcutaneous injection and the animal will be kept under warm conditions (adjacent heating pad) and under supervision until full recovery. The animal will then be returned to the animal housing room and provided food and water ad libitum. The animals will be maintained for 4 weeks to measure changes in vessel wall remodeling induced by AMPK alpha ablation with/without restoration of AMPK signaling with CA-AMPK. The animals will then be sacrificed, and the appropriate tissues obtained for subsequent analyses.

3. Method of wound closure

a. Number of layers

The subcutaneous tissue will be closed first using sterile 6-0 Prolene blue monofilament suture (non-absorbable; Ethicon), followed by closure of the skin using sterile standard rodent wound clips. This equals two layers of wound closure.

b. Type of wound closure and suture pattern:

For sub-dermal sutures, an interrupted suture pattern will be used.

c. Suture type/size / wound clips/tissue glue:

6-0 absorbable suture (Ethicon); standard 9 mm sterile rodent wound clips (skin clips).

d. Plan for removal of skin sutures/wound clips/etc:

Wound clips will be manually removed (with a standard rodent wound clip remover) after 7-10 days if they do not spontaneously fall off; however, by this timepoint most if not all clips will have fallen off unassisted.

F. Anesthetic Protocol:

If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary.

	Agent	Concentration	Dose (mg/kg)	Volume	Route	Frequency	Duration
Pre-emptive analgesic	Meloxicam	5 mg/mL	5-10 mg/kg BW	25 uL	PO	Once prior to surgery; 1/24 hours afterwards	day of and day after surgery, then as needed

						as needed.	
Pre-anesthetic							
Anesthetic	DCM cocktail for mice	ketamine (90 mg/ml), xylazine (10 mg/ml)	90 mg/kg and 10mg/kg	0.1 ml / 10 g BW	IP	1 (prior to surgery); supplemental doses (10% original dose) as needed	Throughout the operative procedure in order to ensure adequate anesthesia
Analgesic Post Op	Meloxicam	5 mg/mL	5-10 mg/kg BW	25 uL	PO	1/24 hours as needed.	
Other	Lidocaine		4 mg/kg BW	~50 ul	topical	2x during surgery	

1. Criteria to monitor anesthetic depth, including paralyzing drugs:

Breathing depth and volume; tail pinch and/or toe pinch reflex.

2. Methods of physiologic support during anesthesia and immediate post-op period:

If animal has difficulty in breathing during recovery, then the body will be supported in a semi-upright position (to aid breathing) through use of sterile gauze and bedding. Animal body temperature will be maintained with use of a heating lamp located approximately 12 inches above the animal and separated by a surgical blanket. Also, supplemental airflow directly into the nose and mouth (via tubing) will aid in breathing. Supplemental fluids will be given as stated above (4.19.E.2).

3. Duration of recovery from anesthesia (immediate post-op period):

Animals are usually sternally recumbent within 2 hours following anesthesia.

4. Frequency/parameters monitored during immediate post-op period:

Depth and ease of breathing; whisker movement, eye movement; ambulation at later stages of recovery; potential wound dehiscence/bleeding will be checked every 3-5 minutes until animal is ambulatory followed by checking every 30 minutes until animal is returned to vivarium.

5. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort during the immediate post-op period:

Pain and discomfort will be controlled by taking the following precautions. Prior to surgery the area of interest will be cleansed with an antimicrobial/antibiotic solution to prevent contamination of the exposed tissues. All surgical equipment that will be used will be thoroughly cleaned and disinfected through autoclaving prior to use. Instruments between surgeries (between animals) will be sterilized with a glass bead sterilizer. Only sterile suture will be used. During surgery, supplemental oxygen will be provided if dyspnea is observed, and supplementary anesthetic will be provided as needed. An effective analgesic will be provided to the animals immediately before surgery and then as needed during surgery and recovery. Discomfort associated with surgery-induced dehydration will be minimized through the provision of supplementary fluids given to the animals during the surgery as well as immediately following the surgery. To prevent dehydration of the eyes during the surgery and recovery, an optical ointment will be placed on the opened eyes of the animal. Lidocaine will be applied topically to the exposed vessel to enhance vascular relaxation and also to induce local anesthesia.

6. List criteria used to determine when animals are adequately recovered and when the animals can be returned to their home environment:

When animals become sternally recumbent and are mobile and can access food and water.

G. Recovery from Surgical Manipulations (after animal regains consciousness and is returned to its home environment)

1. What parameters will be monitored:

The surgery site will be monitored for bleeding, swelling, and dehiscence and the depth and frequency of breathing, ease of mobility/ ambulation, eating and drinking frequency, and overall animal well-being will be monitored post-surgery.

2. How frequently will animals be monitored:

Animals will be continually monitored during recovery period until normal eating/drinking habits and mobility/ambulation are achieved, and thereafter on a daily basis. The following signs of distress will be noted (and if overtly evident, early euthanasia performed): immobility, huddled (hunched) posture, inability to eat or drink, ruffled fur, self-mutilation, vocalization, wound dehiscence, hypothermia, or greater than 20% weight loss.

3. How long post-operatively will animals be monitored:

Usually surgeries are performed in the morning hours, and animals then remain under close supervision for the remainder of the day (~5-7 hours) before returning to the animal housing facility. After this period of time animals are normally fully recovered from surgery. After this animals will be checked daily for 4 weeks.

H. Surgical Manipulations affecting animals

1. Describe any signs of pain/ discomfort/ functional deficits resulting from the surgical procedure:

The only functional deficit resulting from this procedure is occasional closure of the ipsilateral eye.

2. What will be done to manage any signs of pain or discomfort/ (include pharmacologic and non-pharmacologic interventions):

An analgesic will be administered to manage surgical pain or discomfort.

3. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Discussed above

5. Euthanasia

**Please refer to the 2007 AVMA Guidelines on Euthanasia and DCM Guidelines to determine appropriate euthanasia methods.*

5.1 Euthanasia Procedure. If a physical method is used, the animal will be first sedated/anesthetized with CO_2 or other anesthetic agent. If prior sedation is not possible, a **scientific justification** must be provided. All investigators, even those doing survival or field studies, must complete this section in case euthanasia is required for humane reasons.

Generally, the euthanasia method will first involve an overdose (2-2.5x dose used for surgical anesthesia) with an overdose of ketamine/xylazine for mice until breathing has ceased. Immediately after cessation of breathing a pneumothorax with exsanguination will be performed. For breeding adults and weanlings, CO2 euthanasia will be performed, and for neonates/juvenile animals isoflurane will be used when needed.

5.2. Method of ensuring death (can be a physical method, such as pneumothorax or decapitation for small species and assessment method such as auscultation for large animals):

Death is ensured through pneuomthorax (open chest) after cessation of breathing (under anesthesia). For neonates, death ensured by decapitation.

5.3. For field studies, describe disposition of carcass following euthanasia (If carcass will be kept for genetic/morphological/phylogenetic analysis, please include preservation, transportation, and storage technique):

I acknowledge that humane care and use of animals in research, teaching and testing is of paramount importance, and agree to conduct animal studies with professionalism, using ethical principles of sound animal stewardship. I further acknowledge that I will perform only those procedures that are described in this AUP and that my use of animals must conform to the standards described in the Animal Welfare Act, the Public Health Service Policy, The Guide For the Care and Use of

Laboratory Animals, the Association for the Assessment and Accreditation of Laboratory Animal Care, and East Carolina University.

Please submit the completed animal use protocol form via e-mail attachment to iacuc@ecu.edu. You must also carbon copy your Department Chair.

PI Signature: _____

Date: June 26, 2012

Veterinarian: _____ Date: 7/3/12

IACUC Chair: _____ Date: 7/3/12

APPENDIX 1 - HAZARDOUS AGENTS			
Principal Investigator: David Tulis	Campus Phone:252-744-2771		Home Phone:252-353-5957
IACUC Protocol Number:	Department: Physiology		E-Mail: tulisd@ecu.edu
Secondary Contact: Joshua Stone Department: Physiology	Campus Phone:744-3662	Home Phone: 828-231-8222	E-Mail: stonej09@students.ecu.edu
Chemical Agents Used: CA-AMPK	Radioisotopes Used:		
Biohazardous Agents Used: replication deficient recombinant adenovirus (15uL/animal)	Animal Biosafety Level:	Infectious to humans? NO	
PERSONAL PROTECTIVE EQUIPMENT REQUIRED: STANDARD PERSONAL PROTECTIVE EQUIPMENT FOR ALL ANIMAL CARE PERSONNEL			
Route of Excretion: mostly absorbed into tissues, minimal amount excreted in urine and feces			
Precautions for Handling Live or Dead Animals: wear personal protective equipment			
Animal Disposal: all animal material will be placed in biohazard bags for disposal			
Bedding / Waste Disposal: bedding placed in municipal trash			
Cage Decontamination: normal cage washing will be sufficient			
Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment: ENSURE THAT STANDARD PERSONAL PROTECTIVE EQUIPMENT IS WORN BY ALL ANIMAL CARE PERSONNEL			
Initial Approval Safety/Subject Matter Expert Signature & Date _____			

Davenport, Janine

From: Capehart, Anthony
Sent: Thursday, June 28, 2012 3:51 PM
To: Rosenbaum, Matthew; Davenport, Janine
Subject: RE: memo request

It was the 192 vs 240 for the expts that was throwing me. I'm good with the clarification as given as long as it's appended to the AUP. Thanks,

Tony

From: Rosenbaum, Matthew
Sent: Thursday, June 28, 2012 3:48 PM
To: Davenport, Janine; Capehart, Anthony
Subject: RE: memo request

Works for me.

Matt Rosenbaum DVM, MS, DACLAM

From: Davenport, Janine
Sent: Thursday, June 28, 2012 3:47 PM
To: Capehart, Anthony
Cc: Rosenbaum, Matthew
Subject: FW: memo request

Is this what you need?

From: Tulis, Dave
Sent: Thursday, June 28, 2012 3:45 PM
To: Davenport, Janine
Cc: Stone, Joshua Daniel
Subject: RE: memo request

Janine, got a few minutes so I thought I'd write back per your question below.

The discrepancy between the animal numbers on the AHA grant (240) and the numbers on the AUP (896) is simple to explain: on the AHA grant Josh only included the numbers of animals to be used in the actual experiments and did not include estimates of animal numbers needed for breeding purposes. The AUP includes both animal numbers needed to conduct the experiments (192) and the numbers needed for breeding (704). One more observation: the animal numbers needed to conduct experiments on the grant (240) and on the AUP (192) do not match exactly – this is because during calculation of animal numbers needed for breeding, it became apparent to us that we could use some of those "breeding" animals as WT controls, and so this reduced somewhat the animal numbers anticipated to be needed for experimental purposes on the AUP (in other words, it removed some of the "control" animal numbers from the originally estimated 240). I hope this helps to clarify this issue – if not, and I know it's a bit confusing, please let me know – thanks!

Also, yes indeed, this is JIT for a newly awarded AHA Pre-doc grant for Josh! Thanks again,

Sincerely,
dave

From: Davenport, Janine
Sent: Thursday, June 28, 2012 1:55 PM
To: Tulis, Dave
Subject: memo request
Importance: High

Dr. Tulis –

The IACUC review process has begun on your protocol and grant. The protocol has gone out to the committee and they have 3 working days to make comments. The only comment now is 'pending IBC approval'.

For the grant, they are requesting a brief memo clarifying numbers – see below. Please send in a memo, do not change the AUP. Any changes to the protocol at this point would have to go back to the committee.

> -Numbers of animals for grant (240) are difficult to reconcile with those in the the AUP (896). If this is JIT for AHA and time is critical, maybe just a quick clarification in a memo that can be attached to the AUP to indicate exactly which animals in the AUP will be used for the grant.

Thanks,
Janine

Davenport, Janine

From: Taylor, Yvonne
Sent: Friday, June 29, 2012 3:55 PM
To: Tulis, Dave
Cc: Johnson, Edward Harvey; Chaplinski, Nicholas Joseph; Smith, Charles Jeffrey; Lust, Bob; Davenport, Janine; McRae, Susan; Aycock, Dale
Subject: Biological Safety Registration
Attachments: Amendment Approval.pdf

Dr. Tulis,
Please see the attachment regarding your Biological Safety registration.
Thank you,

Yvonne B. Taylor
ECU Office of Prospective Health
Brody School of Medicine
Mailstop 640
600 Moye Blvd. LSB 188
Greenville, NC 27834
252-744-2070
252-744-2417 (fax)

The Brody School of Medicine
Office of Prospective Health
East Carolina University
188 Warren Life Sciences Building • Greenville, NC 27834
252-744-2070 office • 252-744-2417 fax

Occupational Medicine
Employee Health

Radiation Safety

Infection Control

Biological Safety

TO: Dr. David A. Tulis
Department of Physiology

FROM: Eddie Johnson/Nick Chaplinski NSC
Biological Safety Officers

RE: Registration Amendment Final Approval

Date: June 29, 2012

Your Biological Safety Protocol, Tulis DA, 08-01 "**NO-independent cGMP regulation of vascular remodeling in mice**" has been given administrative **approval** to add C57BL/6J background; homozygous "flox" AMPK alpha1 (Prkaa1) and alpha2 (Prkaa2) mutant mice; hemizygous smooth muscle (SM)-specific Cre-recombinase-expressing mice to be conducted at Biosafety Level ABSL 2 in Brody 6E-108 based on your registration/revisions submitted,

using: A. Biohazards

- ☐ Infectious Agent(s)
- ☐ Biotoxin(s)
- ☐ Allergen(s)
- ☐ Prion(s)
- ☐ Human blood, fluid, cells, tissue or cell cultures
- ☐ Transformed cells
- ☐ Other

and/or B. ☐ NIH Use of Recombinant DNA (or RNA) molecules, microorganisms use or breeding transgenic or techniques (plasmids, viral vectors, transfection); of transgenic animals or plants at NIH Category .

This approval is effective for a period of 3 years and may be renewed with an updated registration if needed at that time. Your laboratory will be inspected periodically (every 1-3 years) depending upon the materials/techniques used.

Please notify the Animal Care staff before beginning work with Biohazard agents in animals. Also please keep in mind all individuals who will be exposed to or handle human-derived biohazardous agents will be due for Blood Borne Pathogens refresher training annually.

Please do not hesitate to contact Biological Safety at 744-2070 if you have any questions, concerns, or need any additional information. Best wishes on your research.

cc: Dr. Jeff Smith, Chair, Biosafety Committee
Dr. Robert Lust, Chair
Janine Davenport, IACUC
Dr. Susan McRae, IACUC
Dale Aycock, Comparative Medicine