

Protocol Detail Report

Printed By: Murphy, Angela
3/4/2019 8:03:48 AM

Report Comments

Protocol Information

Version # 6

Reference Number 100482

Protocol Number 2044-100482-093011

Protocol Title Inflammation in high fat diet induced obesity: Benefits of quercetin

Protocol Type: Amendment

Principal Investigator: Murphy, Angela

Approval Date: 5/8/2014

Submittal Date: 5/8/2014

Effective Date: 5/8/2014

Author: McClellan, Jamie

Renewal Date: 9/30/2014

Status: Approved

Next Review Date: 9/30/2014

Inactive Date: 6/4/2014

Expiration Date: 9/30/2014

Amendment Information

1

Amendment Justification

1.1

Briefly explain in language understandable to a layperson the need to amend this AUP.

We need to change the fund number.

Type of Amendment

1.2

Please select all that apply.

Change in Personnel or Other Administrative Change

1.2.1

Change in Personnel or Other Administrative Change

Below is the approved AUP.

1.3

Please make your requested changes in the appropriate fields.

Administrative

2

Reference Number

2.1

This is a system-generated number used to identify your request. This is not the AUP number. The AUP number will be assigned when the protocol is approved.

100482

Principal Investigator

2.2

Murphy, Angela

Angela.Murphy@uscmed.sc.edu

(803) 216-3414 ext

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Protocol Associates

2.7

Please list all personnel that will participate in the work proposed. Use the green plus sign to the right to add Protocol Associates. If the person is not on the list, please contact DLAR at 777-8106. Under "Comments", please summarize the experience/training of the individual as it relates to his/her duties on this proposal.

Carson, James

Responsibilities Will oversee aspects of the protocol including tissue collection and nutritional stimulation.

Comments Dr. Carson has 15+ years of experience working with mice to investigate skeletal muscle biology, including tissue collection and nutritional stimulation.

Co-Investigator **Key Associate** **Authorized to Order Animals**

Cranford, Taryn

Responsibilities Will perform body fat analysis, animal monitoring and tissue collection.

Comments Has 1 year experience working with mice on similar protocols including body fat analysis, animal monitoring, and tissue collection.

Co-Investigator **Key Associate** **Authorized to Order Animals**

Enos, Reilly

Responsibilities Will perform body fat analysis, animal monitoring, non-survival surgery, insulin and glucose tolerance tests, special diet administration and tissue collection.

Comments Has 5 years experience working with animals including body fat analysis, animal monitoring, non-survival surgery, insulin and glucose tolerance tests, special diet administration and tissue collection.

Co-Investigator **Key Associate** **Authorized to Order Animals**

Hardee, Justin

Responsibilities Will conduct non-survival tissue collection and nutritional stimulation.

Comments Has worked with mice for 2 years and assisted with non-survival surgeries for 2 years as well. Was trained by Dr. Carson and Melissa Puppa on tissue collection and nutritional stimulation.

Co-Investigator **Key Associate** **Authorized to Order Animals**

Hetzler, Kimbell

Responsibilities Will conduct non-survival tissue collection and nutritional stimulation.

Comments Has worked with mice for 2 years and assisted with non-survival surgeries for 2 years as well. Was trained by Dr. Carson and Melissa Puppa on tissue collection and nutritional stimulation.

Co-Investigator **Key Associate** **Authorized to Order Animals**

McClellan, Jamie

Responsibilities Will perform eyebleed procedure, non-survival surgery, fecal transfer and tissue collection.

Comments Has worked with mice on previous protocols of the same nature over the past 5 years. Has 5 years experience in animal exercise protocols, eyebleeds, non-survival surgery, gavage and tissue collection.

Co-Investigator **Key Associate** **Authorized to Order Animals**

Murphy, Angela

Responsibilities Will perform tissue collection.

Comments Has worked with mice on previous protocols of the same nature over the past 10 years. Has over 10 years of experience in animal exercise protocols and tissue collection.

Co-Investigator **Key Associate** **Authorized to Order Animals**

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Velazquez, Kandy

Responsibilities Will perform body fat analysis, animal monitoring, eyebleeding, glucose and insulin tolerance tests, fecal transfer, diet administration and tissue collection.

Comments Has 8 years experience working with mice on similar protocols including body fat analysis, eyebleeding, animal monitoring, glucose and insulin tolerance tests, fecal transfer, diet administration and tissue collection.

Co-Investigator

Key Associate

Authorized to Order Animals

Created By 2.8

McClellan, Jamie - INACTIVE

843-319-6696 ext

Continuation 2.9

Is this submission a continuation of a previously approved AUP?

Yes

No

2.9.1

Yes

2.9.2

No

Project Information 3

Title 3.1

Enter the project title.

Inflammation in high fat diet induced obesity: Benefits of quercetin

Extramural Funding 3.2

Is this project supported by current or pending extramural funding?

Yes

No

3.2.1

Yes

3.2.2

No

Study Objectives 4

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Authorized Amounts

5.1.1.3

Use the green plus sign to the right to add animal numbers by USDA Pain and Distress Categories. You can add more than one stress category by pushing the green plus sign again. For definitions of the USDA Pain and Distress Categories, use the help button to the right of each category. This section should include ALL animals to be used, ordered, bred, etc.

Pain Category	Authorized	Requested	On Order	Received	Adjustment	Available
USDA Category C	320	0	0	0	0	320
Totals	320	0	0	0	0	320

Category E

5.1.1.4

Will animals be placed in Category E? Category E is for animals that are subjected to painful or stressful procedures without the use of anesthetics, analgesics, or tranquilizers. Withholding of anesthetics, analgesics, or tranquilizers can only

be allowed if it is scientifically justified in writing and approved by the IACUC. Examples of category E procedures are lethal dose studies (e.g. LD50 studies) that allow animals to die without intervention, pain studies that would not be possible if pain-relieving agents were administered, and psychological conditioning experiments that involve painful stimuli such as a noxious electrical shock that cannot immediately be avoided by an animal.

Yes

No

5.1.1.4.1

Yes

5.1.1.4.2

No

Animal Number Justification

5.1.1.5

Attachments: TABLE 1 6.7.13.docx Aims 3-7 Tables 3.17.14.docx

Provide a detailed justification for the number of animals used over the course of study or the three year approval period. Include a description of each experimental and control group of animals. The use of tables or charts is recommended to explain each group. Use the paperclip symbol to the right to attach documents. Explain how groups sizes were determined. A power analysis should be used to substantiate the numbers requested.

The sample size was conservatively estimated based on expected effect sizes. Specifically, we assumed a standard deviation of 1.0 and a minimum detectable difference in means of 1.5. The power for detecting an effect based on these values is 95%; we maintain 91% power even if 2 mice per group drop out (i.e. have to be removed from the experiment). We have some very interesting results so far and would like to repeat this study to continue research in this area. We have shown gender differences in the inflammatory response to high fat diet induced obesity and we will now examine possible gender differences in the effects of quercetin in this model. So we are requesting an additional 80 mice for this protocol. The severely obese mice that we received from Jackson were of no use to the previous investigator and they were sent to us to be able to make use of them before sacrifice. We were given 50 high fat diet mice and 15 age-matched controls mice. We have come up with 4 studies to utilize all 65 of these mice and have reasonable group numbers for pilot data for a grant submission.

Housing Site(s)

5.1.1.6

Please indicate the site(s) where animals will be housed. Use the green plus sign to the right to add housing site(s).

GSRC

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Chemical Euthanasia Agents

5.1.1.10

If a chemical agent is used for euthanasia, please specify the agent, the dosage and the route of administration. Use the plus sign to add rows. Scroll all the way across the screen to fill in all sections.

Agent	Dosage	Route	Source (Must be pharmaceutical grade)
Isoflurane	overdose	inhalation	ARF

AVMA Guidelines

5.1.1.11

Attachments: <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

If the method(s) of euthanasia include those not recommended by the AVMA Panel Report on Euthanasia (e.g., decapitation or cervical dislocation without anesthesia), provide scientific justification why such methods must be used. A link to the AVMA Panel on Euthanasia is provided.

Procedures

5.1.1.12

Standard Procedures are procedures that are routinely performed in research animals, such as injections, administration of special diets, behavioral studies, exercise studies, etc. These procedures should be described in the above "Experimental Design" section with enough detail that the experimental design can be understood. Here, you should provide the specifics. If a procedure you wish to perform is not included in the list, then use the "Non-Standard Procedures" section for that procedure. Use the green plus sign to the right to add Standard Procedures.

5.1.1.12.1

Imaging

Location

5.1.1.12.1.1

Please select the room(s) where the procedure will be performed.

GSRC Surgery Suite 007

5.1.1.12.1.1.1

GSRC Surgery Suite 007

Procedure Description

5.1.1.12.1.1.2

Please describe in detail the procedures to be performed on laboratory animals. Please do not include any details about scientific methods that do not directly involve the use of animals (such as assays performed on tissue or blood obtained from animals or measurements on the materials to be administered-that information should be included in the Experimental Design section).

The mouse is anesthetized using an EZ-Anesthesia machine. The mouse is initially anesthetized in the chamber with 1.5-2% isoflurane in oxygen. Then the mouse is transferred to the platform on the DEXA scanner. The mouse is kept under anesthesia using a nose cone with the same dose of isoflurane throughout the scan. The scan lasts 5 minutes after which the mouse is returned to the cage to recover.

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Surgical Procedure

5.1.1.14.1.
2.1.8

Completely describe the surgical procedure.

Insulin signaling: Mice will be given an subq injection of ketamine/xylazine/acepromazine. Partial muscle and liver will be excised and cauterized to prevent the animal from bleeding out. Then mice will be given an ip injection of insulin (5units/kg) and after 5 minutes, the rest of the tissues will be removed. Liver, fat, and muscle will be dissected before excising the heart.

Tissue Collection: All animals will be anesthetized by a ketamine-xylazine-acepromazine cocktail (1.4 ml per kilogram body weight) and hindlimb skeletal muscles (soleus, plantaris, gastrocnemious, tibialis anterior, EDL), epididimal fat pad, and heart spleen will be excised and snap frozen in liquid nitrogen for further analysis. Tibias will also be removed as a correction factor for body size. The small and large intestine will be removed, flushed with PBS, opened longitudinally, flattened with cotton swab, and fixed in 10% buffered formalin (Fisher) for 24 hrs. The animal will be euthanized by heart removal. After the animal is anesthetized, an incision will be made at thoracic level and the diaphragm and rib cage dissected to expose the heart.

Non-Survival Surgery Euthanasia

5.1.1.14.1.
2.1.9

Please describe how the animal(s) will be euthanized after surgery.

The heart will be removed from the anesthetized mouse after collection of the tissues to achieve euthanasia.

No

5.1.1.14.
1.2.2

No

5.1.1.14.
2

Restraint

5.1.1.15

Will animals be more than momentarily restrained for any procedures as part of this proposal?

Yes

No

5.1.1.15.
1

Yes

5.1.1.15.
2

No

Food and/or Water Restriction

5.1.1.16

Will access to food or water be restricted as part of this proposal?

Yes

No

5.1.1.16.
1

Yes

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Food and/or Water Restriction Description

5.1.1.16.1
1

Attachments: Special Diet SOP.pdf

Please describe precisely the duration and frequency of food/water restriction. Please follow the attached Institutional Policy.

Mice will be fasted for 5 hours during daylight hours prior to blood collection and tissue collection. We have chosen this short fast during a time when the mice are not eating much so as to stress them as little as possible. Only their food will be removed. The mice will still have access to water.

Food and/or Water Restriction Justification

5.1.1.16.1
2

Please provide justification for food/water restriction. Please detail the methods to be used for monitoring the animals to assure that they do not experience unnecessary pain or distress during food/water restriction.

The purpose of this study is to examine the benefits of quercetin on high fat diet induced obesity. Some of the biochemical markers that we need to measure require that blood be collected in the fasted state such as insulin, glucose, and cholesterol. Mice will be monitored for pain or distress hourly during the 5 hour fast.

The purpose of aims 3-7 is to study the effects of severe high fat diet induced obesity on macrophage induced inflammation. To measure metabolism and protein turnover, blood and some tissue need to be collected in the fasted state.

5.1.1.16.
2

No

Removal from the Department of Laboratory Animal Resources

5.2

Will animals ever be removed from DLAR for longer than 12 hours?

- Yes
 No

5.2.1

Yes

5.2.2

No

Hazardous Agents

5.3

Will hazardous agents be used in this protocol? This includes infectious agents, carcinogens, toxic chemicals, radioisotopes and recombinant nucleic acids.

- Yes
 No

5.3.1

Yes

Hazardous Agents

5.3.1.1

Attachments: MSDS_DSS.pdf

Please select ALL hazardous agents used in these experiments.

Dextran sulfate sodium (DSS)

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Principal Investigator's Assurance Statement

10.1

These assurances must be certified by the Principal Investigator; an E-signature will be required. THE E-SIGNATURE MUST BE SUBMITTED BY THE PI WHEN THE PROTOCOL IS SUBMITTED.

I assure that all the information contained in this Animal Use Proposal is true and all the animal procedures described for this study accurately summarize the nature and extent of the proposed use of animals. If this project is to be funded by extramural source, I further assure that this proposal accurately reflects all procedures involving laboratory animals described in the grant application to the funding agency.

I agree to abide by the provisions of the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press), the Animal Welfare Act (PL 89-544 and Amendments), and the University of South Carolina policies for the care and use of animals.

I certify that this project does not unnecessarily duplicate previously reported experimental work.

I assure that every effort has been made to minimize the number of animals used.

I assure that every effort has been made to reduce the amount of pain, distress, and/or discomfort these animals must experience. I understand that if I cannot be contacted in the event that animals in this project show evidence of distress, illness, or pain, emergency care, including euthanasia if necessary will be administered at the discretion of the veterinary medical staff.

I will promptly notify the IACUC regarding any unexpected study results that impact the health and well-being of the animals.

I am aware that no change(s) to the final approved proposal may be initiated without prior written approval from the IACUC.

I understand that approval of this proposal is for a maximum of three (3) years. If animal work on the project is to continue beyond three years, a new Animal Use Proposal must be submitted. An annual review is required.

I agree.

I do not agree.

10.1.1

I agree.

10.1.2

I do not agree.

Office Use Only

11

Co-Author

11.1

Admin, IACUC

iacuc@mailbox.sc.edu

777-8106 ext

Does the AUP contain surgical procedure(s)?

11.2

Yes

Does the AUP contain multiple survival surgical procedures?

11.3

No

Does the AUP contain prolonged physical restraint?

11.4

No

Does the AUP include food/water restriction or deprivation?

11.5

Yes

Does the AUP involve the use of hazardous agents?

11.6

No

