Section A. Administrative Data: (Link)

1. Title of Project: The role of the ovary in metabolic function

2. Estimated Start Date: 6/1/2010 Estimated Completion Date: 05/31/2013

3. Type of Protocol. Complete each line.
   a. Initial submission [ X] 3 Yr Renewal [] Modification [ ]
   b. Previous protocol number if 3 Yr renewal or Modification: NA [ ]
   c. Field Study: YES [ ] NO [X ] If YES, attach required permits or provide documentation that permits are not required.
   d. Location of Research: College Park: YES [X] NO [ ]; Other-than UMD: YES [ ] NO [X]
   e. Location if research is conducted away from UMD: NA [ X]

4. Principal Investigator.
   Name: Espen E Spangenburg, Ph.D. Department: Kinesiology
   UMD Address: 2134A SPH Office phone: 5-2483 Lab phone: 5-4579
   Fax: 5-5578 E-mail: espen@umd.edu University Title: Assistant Professor

5. Key Personnel. List name, role*, university address, phone and e-mail.
   Lindsay Wohlers (GS) 2128 SPH Bldg kacampbe@umd.edu 301-405-4579
   Kathryn Campbell (GS) 2128 SPH Bldg lwohlers@umd.edu 301-405-4579

   * Indicate role of personnel as CO (co-investigator), CS (collaborating scientist), PD (post-doctoral)
   US (undergraduate student), GS (graduate student), T (technician).

6. Has or will this proposal be submitted through ORAA? YES [ X] NO [ ]
   If applicable -
   a. ORAA Proposal ID number(s): unknown
   b. Title of associated proposal(s) or award(s): unknown not submitted yet
   c. Name of the PI on the proposal or award application: Espen E. Spangenburg
   d. Other key personnel supported under this proposal or award: [ ] NA

7. Funding for animal procurement and care: DRF and submitted grants
(i.e. existing grant, pending grant, submitted grant, departmental, DRF, not yet determined, not applicable)
Type of Grant (i.e. NIH, NSF, Other etc): not yet determined

Section B. Animal Requirements: (Link)

1. Animals. List species; age or weight at use; sex; strain, stock, common or scientific name; source; holding location (bldg) and total number.

Species: Mouse
Age at use: 2 months to 20 months
Sex: Male and Female
Strain: C57/BL6
Stock:
Common or Scientific Name: Mus musculus
Source: Harlan
Holding Location (bldg): CARF
Total Number: 672

2. Description of Animal Usage.
   a) Approximate number of animals used each year: Detailed explanation appears below. Experiment 1 will be done over the course of 2 years. Experiment 2 will be completed in year 2 and experiment 3 in year 3.
   b) Approximate maximum number of animals on hand at any given time: ~130. This will only happen one time. Otherwise we anticipate less than 100 animals to be housed at CARF.
   c) Approximate maximum number of weeks any single animal may be housed: no more than 12 weeks

3. Number of animals by species that will be used in this study that are currently assigned to other protocols: none

   a. Approximate number of rodent pups to be euthanized prior to weaning: NA [X ]
   b. Approximate number of chicks to be used or euthanized immediately post-hatch:
      NA [X ]

Section C. Transportation: (Link) NA [ X ]

Section D. Lay Summary: (Link)
State your study objectives and goals as they relate to the proposed use of animals. Write for nonspecialists and limit to 300 words.

Loss of ovarian function in females is associated with the development of abdominal obesity, increased risk of type 2 diabetes, and the metabolic syndrome. Currently, we have poor understanding of how the ovary affects metabolic function of peripheral tissue. Therefore, the primary goal of this study is to delineate metabolic mechanisms that are altered when ovarian
function is lost in female mice of various ages. In addition, a secondary goal will be to determine if physical activity can be used in a therapeutic sense to overcome the metabolic changes that occur with lost ovarian function. Loss of ovarian function will be induced by either surgical ovariectomy or thoroughly weekly fulvestrant treatment. Further, two models of exercise will performed using voluntary running wheels or a specifically designed rodent treadmill. The purpose of using the two models of exercise is to have an acute bout of exercise (treadmill) and exercise training (wheel running). Male mice will also measured to determine if any sex differences exist between the female mice that have undergone ovary ablation or fulvestrant treatment. It is expected that data obtained from these studies will improve our understanding of the influence of the ovary on mechanisms that regulate metabolic function of peripheral tissue and in addition provide additional data on the use of exercise as a therapeutic interventions for women who are experiencing loss of ovarian function.

Section E. Rationale for Animal Use: (Link)

1. Justify why animals are required over non-animal alternatives:

Due to the invasive nature of the protocol and the amount of tissue required human studies are not possible. One of the major tissues of interest in this study is the visceral fat pad, which cannot be obtained in humans without a highly specialized surgeon and an extremely invasive surgical procedure. Cell culture is also not an option since it is not possible to mimic age or exercise using cell culture models.

2. Justify appropriateness of species selected:

Mice are an appropriate species because we have shown in preliminary and published studies that using different means to disrupt the ovarian function in the mouse results in metabolic changes typically seen in humans that are post-menopausal or undergoing treatment for breast cancer. Other species of animals are not always suitable. For example, unlike the mouse, rats become hyperphagic with removal of the ovary which would add a cofounding element to our study design if we used the rat. In addition, mice are naturally strong runners in that will exercise for fairly long durations (5-10km/night) with no intervention, so mice are advantageous to our study design.

3. Justify number of animals to be used:

We have determined in a priori power measure that the minimum number per group needed to maintain statistical power (0.80) for each experiment is 12 animals. For a complete breakdown of each group see section F (see below). We have broken down the whole proposal into three independent experiments for ease of reading. In experiment 1, we determine the effect of age (experiment 1) and ovary status on various biochemical based metabolic measures in adipose, liver, and skeletal muscle tissue. In addition, we will determine the effects of exercise across each age and treatment group. We will house the animals in cohorts, thus we will not have all the groups in CARF at once but instead do the study in smaller groups. In experiment 2, we determine the effect of ovary status on cellular and molecular mechanisms that regulate lipid metabolism in multiple tissues. In experiment 3, we will isolate primary cell lines from each group to determine the
mechanistic function of the targets identified in experiments 1 and 2 on metabolic function in an ex vivo fashion. The total number of animals to be used is 672.

4. Justify duplicative research: NA [X]

Section F. Experimental Design and Animal Procedures:

1. Brief description of experimental design and animal procedures including summary table(s) of experimental groups and the number in each group: (Link)

Ovary dysfunction will be induced either through surgical removal of the ovaries or through weekly injections of fulvestrant. Ovariectomy is the most common model used to study the role of the ovary in women’s health. All of the ovariectomy surgeries will be done at Harlan and the animals will then be shipped to UMD. Fulvestrant is an estrogen receptor antagonist that results in inhibition of estrogen receptors in peripheral tissue without ablation of the ovaries. It is approved for treatment of breast cancer in humans. Weekly injection of mice with fulvestrant results in increases in abdominal obesity and ovary dysfunction (see below for specifics on treatment protocol). The male mice are included as controls to determine if ovariectomy surgery or fulvestrant treatment causes the female mice to adopt a metabolic phenotype similar to male mice.

Exercise training will be accomplished through voluntary wheel running. Mice will run 5-10km/night if exposed to a running wheel. We have 40 running wheels currently in CARF.

The acute exercise protocol will be done on a mouse specific treadmill. The animals will be placed on a treadmill and run at 15 m/min for 30 mins. This is an easy protocol for the mice and we do not anticipate any major issues. There is a mild electrical stimulus to encourage the animals to run. If we encounter a mouse that refuses to run (sits at the back of the treadmill for longer than 15-20 secs), we will remove it from the study. In our hands, we very rarely have mice that are not capable of running at this speed or duration. If the mice are removed from the study due to failure to run the mice will be euthanized as described in Section J.

Three different experiments will be conducted to determine the metabolic effect of lost ovary function or estrogen receptor signaling on metabolic function of peripheral tissue. See table 1 for detail explanations (see below). We have divided the total number of animals into three distinct experiments. We need to do this because each experiment requires us to process the in completely different fashion. In experiment 1, we will perform GTT and ITT measures. In addition, we collect the necessary tissue and snap freeze the tissue. These tissues will be used for metabolomics and biochemical measures. In experiment 2, we collect the necessary tissue and snap freeze the tissue for cell signaling and gene expression measures. We are only planning to perform one age and one time point for these experiments until we can better establish our mechanistic targets. Once they are identified we will follow up in subsequent proposal to address the mechanisms across age and time. Finally, in experiment 3, we isolate the tissue from each group and isolate primary cell lines from the adipose tissue, liver tissue and skeletal muscle tissue. Using the targets we indentify in experiment 2, we will use these cultured cells to determine the critical nature of each target for the regulation of metabolic function. Due to the amount of tissue and completely different processing techniques required for each set of experiments, we will be required to repeat portions of the study three different times.
The summary table appears on the next page.

<table>
<thead>
<tr>
<th>Group Type</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo</td>
<td>18 mo</td>
<td>6 mo</td>
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<tr>
<td>Group 1 - Control Female</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Group 2 - Control Female and Ovariectomy Surgery (2 weeks)</td>
<td>12</td>
<td>12</td>
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</tr>
<tr>
<td>Group 3 - Control Female and Ovariectomy Surgery (4 weeks)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 4 - Control Female and Ovariectomy Surgery (8 weeks)</td>
<td>12</td>
<td>12</td>
<td>12</td>
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<tr>
<td>Group 5 - Control Female and 2 weeks of Fulvestrant Treatment</td>
<td>12</td>
<td>12</td>
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</tr>
<tr>
<td>Group 6 - Control Male</td>
<td>12</td>
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<tr>
<td>Group 7 - Exercised Female</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Group 2 - Chronic Exercise Female and Ovariectomy Surgery (2 weeks)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 3 - Chronic Exercise Female and Ovariectomy Surgery (4 weeks)</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 4 - Chronic Exercise Female and Ovariectomy Surgery (8 weeks)</td>
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</tr>
<tr>
<td>Group 5 - Chronic Exercise Female and 2 weeks of Fulvestrant Treatment</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 6 - Chronic Exercise Female and 4 weeks of Fulvestrant Treatment</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 7 - Chronic Exercise Female and 8 weeks of Fulvestrant Treatment</td>
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<td>12</td>
</tr>
<tr>
<td>Group 8 - Control Female and 2 weeks of Fulvestrant Treatment</td>
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<td></td>
</tr>
<tr>
<td>Group 9 - Control Female and 4 weeks of Fulvestrant Treatment</td>
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<td>12</td>
<td></td>
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<tr>
<td>Group 10 - Control Female and 8 weeks of Fulvestrant Treatment</td>
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<td>12</td>
</tr>
<tr>
<td>Group 11 - Control Female and Acute Exercise Female and Ovariectomy Surgery</td>
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<tr>
<td>Group 12 - Acute Exercise Female and Ovariectomy Surgery</td>
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<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 13 - Acute Exercise Female and 2 weeks of Fulvestrant Treatment</td>
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<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Group 14 - Acute Exercise Female and 4 weeks of Fulvestrant Treatment</td>
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<td>12</td>
<td></td>
</tr>
<tr>
<td>Group 15 - Acute Exercise Female and 8 weeks of Fulvestrant Treatment</td>
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<td>12</td>
<td>12</td>
</tr>
<tr>
<td>subtotal</td>
<td>216</td>
<td>216</td>
<td>120</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Administered substances other than aesthetics and analgesics. NA [ ] (Link)
   a. List substance, dose or concentration, route, volume, frequency, site, and needle size.

**Fulvestrant Treatment:** Animals will be subjected to fulvestrant (Faslodex, Sigma) treatment will be intraperitoneal (IP) injected weekly at a dose of 10 mg/kg of body mass. The treatments will continue for 2, 4 or 8 weeks. IP injections will be administered using a sterile ½ inch 29 gauge needle and solutions will be sterilized before use. We have previously found that this dose is well tolerated in that mice show no outward symptoms of discomfort or stress. In addition, they eat and drink in normal fashion resulting in maintenance of their body weight during treatment.

**Glucose Tolerance Test (GTT) Procedures:** Circulating levels of blood glucose will be measured using a standard glucometer in a subset of animals from each group (n=7/group). We will remove food but maintain access to water 8-12 hours before the experiment. Prior to the start of the GTT the next morning, we will weigh the mice and nick the tail with a sterile scalpel blade at the very end to remove roughly 0.25 cm of the tail. A new sterile scalpel blade will be used for each mouse tested. Baseline blood glucose will be measured using a glucose meter (AlphaTRAK, Abbott Labs). A ~3 µL droplet is required for each measurement. Sterilized D-glucose (200 mg/ml, i.p.) warmed to 37°C will be injected at 2 mg/g body weight in normal saline. Blood glucose will be measured again at 30, 60, and 120 minutes by gentle massage of the tail and spotting the blood onto the glucometer strip. The first drop of blood is discarded to ensure accurate analysis of blood glucose. Mice are monitored throughout for excessive bleeding or other adverse conditions. If for
any reason an adverse reaction is detected the test will be discontinued and the campus veterinarian will be contacted. Following the final test, food is returned and mice are monitored for 2 hours to assure complete recovery. **We will keep a written log of all times food is removed and returned to the animals.**

**Insulin Tolerance Test (ITT) Procedures:** Circulating levels of blood glucose will be measured using a standard glucometer in a subset of animals from each group (n=7/group). We will remove food for 6 hrs prior to experiment but maintain access to water. Prior to the start of the ITT the next morning, we will weigh the mice and nick the tail with a sterile scalpel blade at the very end to remove roughly 0.25 cm of the tail. A new sterile scalpel blade will be used for each mouse tested. Baseline blood glucose will be measured using a glucose meter (AlphaTRAK, Abbott Labs). A ~3 µL droplet is required for each measurement. Animals will then receive 0.75U/kg body weight (approximately 11 units) of bovine insulin (Sigma) via intraperitoneal injection. Intraperitoneal injections will be administered once using a sterile 1/2 inch 29 gauge needle. Blood glucose will be measured again at 30, 60, and 120 minutes by gentle massage of the tail and spotting the blood onto the glucometer strip. The first drop of blood is discarded to ensure accurate analysis of blood glucose. Mice are monitored throughout for excessive bleeding or other adverse conditions. If for any reason an adverse reaction is detected the test will be discontinued and the campus veterinarian will be contacted. Following the final test, food is returned and mice are monitored for 2 hours to assure complete recovery. **We will keep a written log of all times food is removed and returned to the animals.**

b. List and provide justification for any non-pharmaceutical grade substances including anesthetics, analgesic and injectable euthanasia solutions that will be used. NA [X]

All anesthetics (i.e. isoflurane) are pharmaceutical grade.

3. Blood collected from live animals. NA [ ] (Link)
   a. List method, site, volume and frequency.

   Blood will be collected for glucose tolerance tests and insulin tolerance tests via tail snip. These tests require sampling of a single drop of blood over time following an intraperitoneal injection of glucose or insulin. See above response in #2 for specifics of testing procedures.

   b. Identify if terminal bleed. N/A

   c. Will animals be anesthetized (local or general) for blood collection? YES [ ] NO [X]  
      If YES, add to table under Section H.

4. Describe methods of restraint: NA [X] (Link)

5. Survival surgery: NA [ X] (Link) All ovariectomy surgeries will be performed at Harlan and the animals will be shipped to UMD upon recovery from the surgery.
   a. Describe the surgical procedure:
b. Describe aseptic methods:

c. List who will perform the surgery and their qualifications:

d. Describe post-operative care and who will provide it: We will monitor the animals and if we see any situations where the animal appears to sick, not eating/drinking, or stressed we will immediately contact the campus veterinarian.

e. Has major survival surgery been performed on any animal prior to being placed on this study? YES [X] NO [] If yes, justify: The ovariectomy model is the most commonly used animal model to replicate menopause in the women. It is well tolerated by the mice and will be performed by experts at Harlan before being shipped to UMD. We monitor the animals and if we detect any changes in food or water consumption we contact the campus veterinarian immediately.

f. Will more than one major survival surgery be performed on any animal while on this study? YES [ ] NO [X] If yes, justify:

6. Describe anticipated resultant effects: (Link)
The anticipated resultant effects of this study are that ovariectomy and fulvestrant treatments will result in peripheral metabolic alterations that increase visceral adipose tissue mass. The end results of fulvestrant treatment are anticipated to be similar to that ovariectomy, however, the underlying mechanisms may differ. We expect to find an upregulation of cellular or molecular mechanisms that regulate fat storage in the adipose, liver, and muscle tissue after ovariectomy and fulvestrant treatments. In addition, we expect that exercise training will prevent the accumulation of lipid by either inhibition of mechanisms that regulate lipid storage or by increasing the ability of the peripheral tissue to oxidize lipid. Finally, we anticipate that the acute exercise bouts will allow us to identify the signals that initiate the therapeutic events of exercise.

7. Describe experimental and humane endpoints: (Link) Include description of pain scoring, by whom, how often, intervention criteria and method to intervene.

All animals will be euthanized and specific tissues will be collected from the animal. None of the experiments will result in any pain or distress for the animal. If animal appears to be in pain, we will remove it from the study and contact the campus veterinarian.

8. Locations (Link)

<table>
<thead>
<tr>
<th>Animal Procedure</th>
<th>Building(s)</th>
<th>Room(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-survival Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euthanasia</td>
<td>SPH</td>
<td>2128</td>
</tr>
<tr>
<td>Tissue Harvesting</td>
<td>SPH</td>
<td>2128</td>
</tr>
<tr>
<td>Behavior Testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Other Experimental Procedures: GTT and ITT

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Species</th>
<th>Number of animals</th>
<th>Category I (Minimal, transient or no pain or distress)</th>
<th>Category II (Pain or distress relieved by appropriate means)</th>
<th>Category III (Pain or distress not relieved)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Collection</td>
<td>Mouse</td>
<td>672</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail Nicks</td>
<td>Mouse</td>
<td>186</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>Mouse</td>
<td>168</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Section G. Pain and Distress:

1. Categorize based on the most severe procedure to which they will be subjected. (Link)

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Species</th>
<th>Number of animals</th>
<th>Category I (Minimal, transient or no pain or distress)</th>
<th>Category II (Pain or distress relieved by appropriate means)</th>
<th>Category III (Pain or distress not relieved)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Collection</td>
<td>Mouse</td>
<td>672</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail Nicks</td>
<td>Mouse</td>
<td>186</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>Mouse</td>
<td>168</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Literature Search for Alternatives to Painful or Distressful Procedures: (Link) Required for procedures under Category II and III to determine if other methods are available that could reduce or eliminate pain or distress.

   a. Sources used (at least 2): PubMed; Google Scholar
   
   b. Date search completed: 04/10/10

   c. Years covered: 1900-2010

   d. Key words used: anesthesia mice isoflurane safety

   e. Summary of the outcome of the search including a statement that no acceptable alternatives were found or why alternatives cannot be used:

   We anesthetize **all our animals (n=672 for the entire study)** with isoflurane (induction 4-5%, maintenance 2-3%) and remove all of the necessary tissues (visceral fat, liver, skeletal muscle, and heart) while the animal is anesthetized. Other alternatives found include using injectable cocktails. These are less advantageous for us due to the accumulation of needles and a number of these drugs are regulated by the DEA, while isoflurane is not. In addition, by using isoflurane we can control the depth and reduce the handling of the mice to ensure lower stress levels. In addition, we do not use CO2 inhalation because a number of our experimental measures require blood flow to remain intact for as long as possible before the tissue is removed. Thus, no acceptable alternatives were found for our chosen form of anesthesia.

Section H. Anesthesia and Analgesia: (Link)

1. List agent, procedure requiring agent, dose, route, and frequency.

   Anesthesia will be performed by inhalation of 4-5% isoflurane induction. After the induction, the isoflurane is lowered 2-3%.  

8
2. Provide details on fasting prior to anesthesia, methods of monitoring anesthesia, and care during anesthesia recovery if not mentioned in Section F: NA []

Food will be removed 4-5 hours prior to anesthesia. The animals will be initially induced in an induction box and then isoflurane exposure will be maintained by using a mouse specific nose cone. The level of induction will be monitored by reflex responses to toe pinches throughout the entire process of the tissue removal. No response to a toe pinch would indicate a sufficient level of induction, if the animal exhibits a toe pinch response we not perform any procedures until the animal reaches a suitable anesthetic plane.

Section I. Biological Materials for Use in Animals: (Link)

1. Will animals be exposed to any of the following materials? This information will help to prevent introductions of infectious agents to University animals.

Animal tissue, fluids or cells YES [ ] NO [X]

2. If YES, explain:

3. Has the material been tested for murine pathogens? YES [ ] NO [ ]

Section J. Animal Disposition and Euthanasia: (Link)

1. Will animals be euthanized? YES [ X] NO [ ]


Animals will be anesthetized via inhalation of 4-5% isoflurane gas and maintained under 2-3% isoflurane. When animals have reached and appropriate plane of anesthesia (as determined by lack of toe pinch reflex), we will remove numerous tissues (heart, liver, skeletal muscle, brain, and adipose tissue) from the mice and snap freeze the tissues. It is important that we use anesthesia since we need the tissues to be blood flow intact. Thus, the animal will be euthanized by exsanguinations, which is a necessity since we remove the heart and diaphragm for subsequent biochemical measures.

3. Method to ensure death: NA [ ]

The tissues to be removed following anesthetization include the heart and diaphragm, such that the animals will be euthanized through exsanguination. The animals will remain anesthetized throughout the entire procedure.

4. Justification for conditionally acceptable or unacceptable methods: NA [X]

5. Method to dispose of euthanized animals: NA [ ]

Final disposal of euthanized animals will be performed via incineration through CARF.

6. Final disposition of animals if not euthanasia: NA [X]
Section K. Hazardous Agents: (Link)

1. Identify all hazardous agents that will be administered to animals.

<table>
<thead>
<tr>
<th>Agents</th>
<th>Check all that apply</th>
<th>List agents &amp; Registration Document # (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radionuclides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological Agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hazardous Chemicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant DNA including recombinant microorganisms and transgenic animals</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Study conducted at Animal Biosafety Level: NA [ X ]

3. Describe precautions and procedures: NA [ X ]

4. Has approval been obtained from the Division of Environmental Safety: Yes [ ] No [ ]

5. Identify any agents administered and expected concentration/activity that will be expelled in animal waste: NA [ X]

6. Identify any agents administered and expected concentrations/activity that will be in animal tissues when the animal is euthanized/disposed: NA [ X ]

Section L. Special Concerns or Requirements: (Link) None [ ]

1. Describe deviations from standard housing and animal care: None [X]

2. Describe special equipment requirements: None [ ]

Voluntary running wheels cages will be used in certain experiments. These cages have already been purchased by Dr. Spangenburg and used in previous studies approved by the UMD IACUC. The cages are already housed at CARF. The other equipment is in the Spangenburg lab.

3. Describe deviations from standard diet including amount and frequency: None [ ]

   Prior to glucose tolerance testing and insulin tolerance testing food and bedding will be removed for 6-12 hours before the test. Upon completion of the test the food will be immediately returned to them.

4. Describe water restrictions: None [X]
5. Describe phenotypes and care of any animals which may be associated with morbidity or shortened lifespan: None [X]

6. Justify any deviations from the Guide for the Care and Use of Laboratory Animals. None [X]

7. Other: None [X]

Section M. Training: (Link) To be completed for each person named in this protocol including the PI.

Name: Espen E. Spangenburg, Ph. D.
Animal activities performed on this protocol: Will assist with tissue removal and oversee the experiments
Credentials: BS, MS, Ph. D.
Experience working with each species: since 1995
Experience with animal procedures listed above: greater than 10 years
Training the individual will receive: none
Year completed UMD PI/Animal User training: 2006

Name: Lindsay M. Wohlers
Animal activities performed on this protocol: Will monitor animals throughout the study, perform all injections and tissue removal
Credentials: BS, MS
Experience working with each species: since 2007
Experience with animal procedures listed above: 2007
Training the individual will receive: No new training is necessary
Year completed UMD PI/Animal User training: 2007

Name: Kathryn M. Campbell
Animal activities performed on this protocol: Will monitor animals throughout the study, perform all injections and tissue removal
Credentials: BS MS
Experience working with each species: 2008
Experience with animal procedures listed above: 2008
Training the individual will receive: No new training is necessary
Year completed UMD PI/Animal User training: 2008

Section N. Principal Investigator Certifications and Acknowledgments:

1. I acknowledge responsibility for the conduct of these procedures and the care of these animals.

2. I will conduct this work with animals in accordance with the protocol as approved by the IACUC and the campus animal care and use guidelines. I will obtain approval from the IACUC before initiating any changes in the protocol.

3. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.

4. I certify that all individuals working on this proposal who have significant animal contact are
participating in the Laboratory Animal Handler’s Medical Surveillance Program.

5. I will maintain appropriate animal records (e.g. census, health, veterinary care, surgery, diagnostic, treatment, etc.)

6. For Category II and III proposals, I certify that I have reviewed the pertinent scientific literature, the sources and/or databases (2 or more) as noted in Section G, and have found no alternatives to any procedures described herein which may cause more than a momentary pain or distress whether it is relieved or not.

7. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under the proposal and have attended training. (Link) Training may include but not be limited to the biology, handling and care of the species, aseptic surgical techniques, research methods that limit the use of animals or minimize distress, proper use of anesthetics and analgesics, and procedures for reporting animal welfare concerns.

Principal Investigator: Signature_______________________ Date ____________

Section O. Concurrences: Protocol number _______ (leave blank) (Link)

O.1. Department Chair certification of approval of resources.

Name: ___________________ Signature: _______________ Date: __________

O.2. Division of Environmental Safety Representative:
Required for studies utilizing hazardous agents. Required prior to approval but not at submission.

Name: ___________________ Signature: _______________ Date: __________

Name: ___________________ Signature: _______________ Date: __________

O.3. Facility Manager or Veterinarian certification of resource capability:

Name: ___________________ Signature: _______________ Date: __________

Facility: ___________________

Section P. Approval:

Certification of review and approval by the IACUC chairperson.

Name: ___________________ Signature: _______________ Date: __________

ARAC Approved – October 8, 2008
Revised –