

PROTOCOL FOR ANIMAL USE AND CAREEmail to: campusvet@ucdavis.edu**CRPRC**

EH&S USE ONLY

**PROTOCOL: 10147
EXPIRES:**

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus monkey	4	CRPRC

Project Title Dietary Restriction and Organ-Specific Energy Expenditure in Rhesus Monkeys

Overnight housing location::	CRPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator (If investigator maintained, attach husbandry SOP's.)		

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Monkeys will have catheters placed in the hepatic vein, femoral vein, and femoral artery and an ultrasound flow probe will be placed around the femoral artery. The animals will be fed an energy restricted diet (60% of ad libitum intake) for a period of two weeks, and blood samples will be collected from the catheters for determination of tissue oxygen consumption.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

The monkeys will be energy restricted (fed 60% of ad libitum intake on standard diet) for a period of two weeks. The animals will wear a jacket to protect the catheters and will be connected to a tether system for blood collections. On two occasions, the animals will be housed for 48 hours in indirect respiration calorimeters for energy expenditure measurements.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

Hazardous Materials (only if in the animal room):

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	CRPRC pilot projects	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Restriction of energy intake (ER) has consistently been shown to increase maximum life span in several animal species. A decrease in oxygen consumption has been proposed as a mechanism for the retardation of aging with energy restriction. To test this theory, it is important to show that ER results in a system wide reduction in organ and tissue oxygen consumption (not just changes in one tissue). The goal of this project is to determine the effect of energy restriction on oxygen consumption in individual organs/tissues. Catheters and remote blood sampling will be used to measure oxygen consumption in the hepatomesenteric bed and hindlimb of adult rhesus monkeys. **Remote blood sampling involves collecting blood through a catheter line in manner such that the animal is not aware the investigator is in the room and sampling blood (the investigator and sampling apparatus are hidden from view).** This study will provide important new information about the influence of energy restriction on oxygen consumption of organs and tissues in an adult primate. The proposed methods may ultimately be used to determine if long-term energy restriction causes a uniform reduction in organ and tissue oxygen consumption.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

<input type="checkbox"/> Monoclonal Antibody Production **	<input checked="" type="checkbox"/> Food or water restriction	<input type="checkbox"/> Special diets; food or water treatment.
<input type="checkbox"/> Polyclonal Antibody Production **	<input type="checkbox"/> Non-recovery surgical procedures	<input type="checkbox"/> Induced illness, intoxication, or disease
<input type="checkbox"/> LD 50 or ID50 studies.	<input checked="" type="checkbox"/> Survival surgical procedures	<input type="checkbox"/> Death as an endpoint (see i below)
<input checked="" type="checkbox"/> catheters, blood collection, intubation	<input type="checkbox"/> Multiple survival surgery	<input type="checkbox"/> Trapping, banding or marking wild animals
<input type="checkbox"/> Prolonged restraint. (8 hrs+)	<input type="checkbox"/> Behavioral modification.	<input type="checkbox"/>
<input type="checkbox"/> Fasting prior to a procedure.	<input type="checkbox"/> Aversive conditioning.	<input type="checkbox"/>

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Four adult male rhesus monkeys (aged 8-20 years) weighing over 10 kg will be used for this experiment. The monkeys will be housed under standard caging conditions with temperature maintained at approximately 21°C and a 12:12 hour light-dark cycle. The animals will be allowed ad libitum access to water throughout the study. The monkeys will be fed a commercial diet (5037 Monkey Diet, PMI Feeds). The animals will be allowed free choice consumption of food during all phases of the study except the two weeks of ER. Food intake will be measured daily and baseline food intake will be calculated as the average daily intake during the first four weeks of the study. During the last two weeks of the study, the animals will undergo energy restriction and will be fed 60% of the average daily food intake determined during the baseline period. During the first four weeks of the study, the animals will be adapted to a jacket and tether system. The tether system will be such that it will not inhibit movement around the total area of the cage. The jackets will remain on the animals through the end of the study. The animals will be studied according to the following schedule:

<u>Week(s)</u>	<u>Assessments</u>
1-8	food intake (daily), health observations (daily), body weight (weekly)
1-4	determination of baseline food intake, jacket and tether adaptation
5	surgery for catheter and flow probe placement
6	baseline calorimetry measurements and measurements of organ oxygen consumption and blood flow
7	start of energy restriction
8	energy restriction, calorimetry measurements, measurements of organ oxygen consumption and blood flow, and removal of catheters
9	monitor animal health prior to return to the colony (monitor for infections or complications with catheter removal)

The monkeys will be divided into two groups of two animals. The start time for the experiment will be staggered by four weeks for each group to prevent overlap in oxygen consumption determinations.

Catheter Placement

On week five of the experiment, catheters will be placed in the hepatic vein, femoral vein, and the femoral artery. Dr. _____ and the CRPRC veterinary staff will perform the surgery. The monkeys will be premedicated with atropine (0.4 mg/kg SC) and ketamine HCl (10 mg/kg IM). After being prepared for aseptic surgery, an endotracheal tube will be placed and the animal maintained on isofluorane gas (1-2% in 2L of oxygen per minute) throughout the surgery. Gas anesthesia will be discontinued as the skin is closed. After a surgical level of anesthesia has been achieved, an incision will be made in the abdomen and a single lumen catheter will be threaded from a branching vein into the hepatic vein. An incision will also be made in the upper thigh and catheter lines will be introduced and threaded into the proximal femoral vein and the femoral artery. After all of the catheters are placed, a small incision will be made in the area between the scapula and a hollow tunneling needle will be used to tunnel the catheters

subcutaneously from the venous or arterial cutdown area to the incision between the scapula. Additional length of the catheters will be coiled and secured within a pouch sewn in the jackets. All catheters will be flushed with heparinized saline (100 U/ml) and capped. To collect blood samples, the catheter lines will be threaded through a flexible metal tether that connects to the back of the jacket. The animals will be chemically restrained with ketamine HCl (15 mg/kg IM) for tether connection or removal. **The tether will be connected to the jacket pocket. The other end of the tether will be connected to a swivel on the top of the cage. Catheter lines will be run from the jacket pocket through the tether and exit the cage through the swivel. The length of the tether will allow the animal to reach all areas of the cage and will not restrict movement.** The catheters will be flushed three times per week with heparinized saline (100 U/ml). At this time, the area where the catheter lines exit the body will be cleaned with chlorohexidine and wiped with a triple antibiotic ointment. **The animals will be housed in squeeze back cages and this procedures can be completed with brief squeeze restraint in the home cage.**

Indirect Respiration Calorimetry

Whole animal oxygen consumption will be measured by indirect respiration calorimetry. The monkeys will be placed in the CRPRC exposure facility calorimetry chambers (same dimensions as home cages) for two consecutive 24 hour measurements. Air will be forced through the chamber at a regulated rate and flow rate will be measured with a mass flowmeter. Chamber exhaust will be continuously sampled for analysis of oxygen content.

Measurement of Organ/Tissue Oxygen Consumption

Approximately 3 ml of blood will be collected anaerobically in heparinized syringes from the catheters in the hepatic and femoral veins and femoral artery. All blood samples will be collected immediately before measurements of blood flow rate at 9:00, 15:00, 21:00, and 03:00. **All blood samples will be collected in the home cage at least one day prior to moving the animal to the calorimeter in the CRPRC exposure facility. A screen will be placed near the cage to shield the investigator from the view of the animal. This will allow blood samples to be collected without causing the animal agitation that may be associated with observing people in the animal room.** Syringes will be immediately analyzed for hemoglobin concentration and oxygen saturation with a hemoximeter. Blood oxygen concentration will be calculated using the following equation:

$$O_2 (\mu\text{mol/ml}) = (A)(B)(59.8)$$

Where A = blood hemoglobin (g/ml), B = percent oxygen saturation, and 59.8 is $\mu\text{mol } O_2/\text{g}$ of hemoglobin. Oxygen consumption will be calculated by the following equations:

$$\text{Hepatomesenteric bed oxygen consumption (mmol/min)} = F_h(C_{fa} - C_h)$$

$$\text{Hindlimb oxygen consumption (mmol/min)} = F_f(C_{fa} - C_{fv})$$

F_h is hepatic vein flow rate (ml/min), F_f is femoral vein flow rate (ml/min), and C_{fa} , C_h , and C_{fv} are the blood oxygen concentrations (mmol/ml) for the femoral artery, hepatic vein, and femoral vein respectively.

Measurement of Regional Blood Flow

Femoral vein blood flow rate will be determined using ultrasound transit time flow probes (2S-Probe; Transonic, Ithaca, NY). While the animals are undergoing surgery for catheter implantation, the flow probe will be placed around the femoral vein between the catheter and the external iliac vein. A hollow tunneling needle will be used to tunnel the probe cable subcutaneously to meet the catheter lines and both will exit the body between the scapula. **At a later time when the tether is connected to the jacket,** a cable connecting the probe to an ultrasonic flowmeter will be threaded through the tether and connected to the electrical leads on the swivel. **The animal will be sedated with ketamine when the tether is connected and the flow probe cable is threaded through the tether.** The leads from the swivel will be connected to the flow meter for all measurements.

Hepatic vein blood flow measurements will be determined by the single

bolus indocyanine green dilution method. **Measurements will be completed on the same day as blood oxygen measurements (two days during the study). Hepatic blood flow measurements will be completed immediately after the 15:00 and 03:00 blood draws on each of the two days.** Dye will be prepared for injection by mixing 25 mg of indocyanine green in 10 ml of sterile saline. Approximately 1 ml of blood will be drawn from the hepatic catheter to serve as a plasma blank. Indocyanine dye solution (1 mg/kg) will then be injected into the femoral vein. Approximately 1 ml blood samples will then be collected simultaneously from the femoral artery and hepatic vein in heparinized syringes at 5,8,11,14 and 17 minutes after injection of the dye. Samples will be centrifuged and the dye concentration determined spectrophotometrically. Blood flow will be calculated by dividing plasma clearance rate by extraction rate.

Plasma clearance rate = volume of dye distribution x slope (from semilogarithmic plot of dye concentration versus time)

Extraction rate = (arterial - hepatic vein dye concentration / arterial dye concentration) x 100

d) **Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require.** The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
1	Energy restriction and catheter placement	All 4	3

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

The species was chosen for the following reasons: 1) Extensive background information exists on the effects of energy restriction on energy expenditure and aging in this species; 2) The size of the species allows the use of arterio-difference measurements of oxygen consumption. These measures are difficult (if not impossible) to perform in rats or other small animals; 3) The investigator has experience with the use of this species in related studies.

The number of animals proposed for use in this experiment represents the minimum number of animals needed to give statistically accurate results based on power calculations using estimated mean changes derived from previous arterio-venous difference studies.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:

Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
rhesus	Ketamine HCl	10	IM	Prior to surgery and when chemical restraint is needed to connect the tether to the jacket (4 times)
rhesus	Isoflurane	1-2% in 2L of oxygen per minute		Surgery (1 time)
rhesus	Buprenorphine	.03	IM	Three times per day for three days post-surgery or as needed after this time.
rhesus	cefazolin	20	IM	Three times per day for three days post-surgery or as needed after this time.

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

The animals will undergo surgery for catheter placement. Following surgery the animals will be monitored for signs of discomfort and administered analgesics as needed. Infections are potential problems with any catheter experiment. Triple antibiotic ointment will be applied (3 times per week) around the catheter exit site to prevent infection. If infection is suspected, a CBC will be taken on blood drawn from a catheter line and any confirmed infection will be treated immediately with antibiotics. The CRPRC veterinary staff will monitor the animals and they have permission to immediately begin treatment if they detect a problem.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

After surgery, all animals will be monitored for signs of discomfort and will be treated with buprenorphine (0.03 mg/kg IM) three times per day for a minimum of 3 days.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

*Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not **unnecessarily** duplicative of other studies.*

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit

http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwood@ucdavis.edu)

What was the date on which you conducted this search?

Searches were completed weekly over the past three years.

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Medline	1966-present	Dietary restriction, energy restriction, energy expenditure, oxygen consumption, organ mass
Current contents	1997-present	Dietary restriction, energy restriction, energy expenditure, oxygen consumption, organ mass

Annual meetings of the ASNS (Nutrition) and GSA (gerontology) and other meetings	1998-present	Discussion with investigators about dietary restriction, aging, and oxygen consumption
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What were your findings with respect to alternative methodologies?

There are currently no suitable alternatives to studying energy restriction at the level of the whole animal. Energy restriction has consistently been shown to increase maximum life span in laboratory rodents. In cell cultures, it is difficult to determine the actual level of energy restriction and few studies have measured the effects of these conditions on life span.

Has this study been previously conducted?

Yes No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) **Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

The animals will not be euthanized

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus	Lethal dose	pentobarbital	60 mg/kg	IV

m) **Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

Catheters will be removed from the animals at the completion of the study and the animals will be returned to the general colony at the CRPRC.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> Withdrawn by Investigator Date of Action: ____/____/____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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