

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

EH&S USE ONLY
PROTOCOL # <u>9735</u>
EXPIRES: _____

Investigator
Last Name:
First:
Middle:
email:
Department:
Phone:
Fax:

Contact
Last Name:
First:
Middle:
email:
Department:
Phone:
Fax:

Species (common names):	Number:	Source:
Rhesus Monkey	24	CRPRC

Project Title Effects of fructose versus glucose feeding on adiposity and metabolism in rhesus monkeys

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

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.

Animals will be fed 500 ml of a 20% glucose or fructose solutions, or a non-caloric sucrolose-sweetened solution per day for up to 5 years. Food intake will be monitored. Adiposity will be measured by DEXA scanning. Metabolic rate will be measured over 24 hours in sealed chambers at the exposure facility. Tissue biopsies will be performed for UCP2 and UCP3 measurements. Insulin sensitivity, hormone and serum lipid levels will be assessed during serial blood sampling.

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.

Animals will be fed 500 ml of a 20% glucose or fructose solutions, or a non-caloric sucrolose-sweetened solution per day. The amount of solution and food consumed will need to be assessed daily.

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 type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input checked="" 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 checked="" 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:	ADA	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8248

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 pctlillman@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.

Obesity is a well-established risk factor for the development of type-2 diabetes. The prevalence of obesity has increased progressively over the past two decades. Dietary intake of fructose has also increased. Whereas glucose stimulates insulin and leptin production, fructose does not. Both insulin and leptin act as long-term peripheral signals of energy status to the central nervous system (CNS), and are involved in the regulation of food intake and energy expenditure. Since fructose administration does not stimulate either leptin or insulin, energy (calories) consumed as fructose essentially are unrecognized by the CNS and the appropriate adjustments of appetite and energy expenditure do not occur. Fructose increases triglyceride levels in both humans and animals. We hypothesize that, over time, a diet high in fructose would promote weight gain, obesity and hyperlipidemia, resulting in an increased risk for type-2 diabetes. To test this hypothesis in a nonhuman primate model susceptible to adult-onset obesity and diabetes, we propose to measure 24-hour circulating leptin concentrations, energy expenditure (by indirect calorimetry), uncoupling proteins (UCP-2 and UCP-3 by fat and muscle biopsies), adiposity (by dual energy X-ray absorptiometry), insulin sensitivity, and serum lipids before and during a 60-month period throughout which rhesus monkeys consume solutions high in fructose or glucose or sweetened with a non caloric sweetner.

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 checked="" 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 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 checked="" 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.)

General Experimental Design: Each animal will be studied at eight different time points during the first year with the procedures described below. The time points for the procedures are defined as follows: a baseline study prior to feeding the high fructose or high glucose solutions, then at 2 weeks, 1, 2, 4, 6, 9, 12 months. Procedures will then be performed every 3 months over the next 4 years.

Dietary Manipulations: Prior to the introduction of the 20% fructose or 20% glucose solution, and during the baseline period, 24 monkeys will receive 500 ml of Sucrolose-sweetened Koolaid in water bottles attached to the cages each day. After the baseline period, 8 monkeys will receive 500ml of 20% glucose koolaid, 8 monkeys will receive 500ml of 20% fructose koolaid and 8 monkeys will receive 500ml of sucrolose-sweetened koolaid each day. Each day, leftover koolaid will be measured, discarded, and replaced with fresh koolaid. The animals' intake of chow biscuits each day will also be measured.

24 Hour Leptin/ Insulin/ Glucose Profiles: To determine the effects of glucose versus fructose feeding on 24-hour circulating leptin, insulin, and glucose concentrations, blood samples will be collected from an indwelling catheter every 2 hours over a 24 hour period on a normal feeding day.

Adiposity: Body weight will be determined to the nearest 0.01 kg twice weekly throughout the studies. Percent and total body fat will be determined under brief ketamine anesthesia by dual energy X-ray absorptiometry (DEXA) TB 175. We will also determine body composition by measuring total body water (TBW) by deuterium dilution (D₂O dilution). Total body water will be estimated from enrichment of the tracer in serum after a 2-hour equilibration period. Following a 12-hour period of withholding food and water, a bolus dose (up to 6 grams) of deuterium oxide tracer will be infused into a cephalic vein via a 23ga butterfly infusion set used to draw the pre-infusion blood sample. Samples for the TBW measurement will be collected at the same time points as samples are collected for the 24-hour leptin measurements. The animals will already be fasted and the dilution tests will coincide with the first two blood draws for leptin. A baseline blood sample (up to 6 ml) will be collected at 0700, the D₂O will be infused, and a second blood sample (up to 6 ml) will be drawn at 0900. Total blood sample volumes for all tests will comply with CRPRC guidelines.

Energy Expenditure: Metabolic rate will be assessed by indirect calorimetry for 24 hours. Cages within sealed chambers used for indirect calorimetry and equipped with flowmeters and instruments for measuring oxygen and carbon dioxide are available at the CRPRC. Monkeys will be acclimated to the chambers with 4-5 training sessions prior to measurement of metabolic rate. The animals will be provided with their usual diet of monkey chow biscuits, KoolAid, and water during testing. We have found that the majority of animals rest quietly in the chambers during the metabolic rate measurements.

Energy expenditure will be measured by the doubly-labeled water method. Two stable isotopes of water, deuterium (2H₂O) and oxygen-18 (H₂ 18O), will be administered IV (antecubital vein) at doses of 136 mg 2H₂O/kg body and 170 mg/kg H₂ 18O. Equilibration within the total body water pool will be verified in 4 ml blood samples taken from antecubital vein at 1,2,3 and 4 hours post-injection. Assessment of the subsequent rates of disappearance of the isotopes from the body fluid will be assessed in 4 ml blood samples taken once/day for the next 10 days. This procedure will be performed 2 times during baseline (prior to the introduction of the sugar solutions) and then 6 and 12 months later.

Tissue biopsies will be performed to allow measurement of uncoupling proteins UCP-2 and UCP-3 in fat and muscle. These uncoupling proteins have an effect on energy expenditure. Biopsies will be performed three times in year one and annually in years two through five. A 1.0 g fat biopsy will be obtained from the interscapular region. A 0.5 g muscle biopsy will be obtained from an epaxial muscle in the same region. All procedures will be performed under ketamine anesthesia (10-15 mg/kg IM).

Glucose Tolerance/Insulin Sensitivity: We will use a fast sampled intravenous glucose tolerance test (FSIVGTT)(Kemnitz et al, 1993). Blood pressure and heart rate will be measured with a cuff instrument

(Critikon) prior to and periodically throughout the FSIVGTT. For the FSIVGTT, animals will be anesthetized with ketamine (15 mg/kg IM) and diazepam (0.1 mg/kg IM) and maintained with a slow ketamine infusion (0.05-0.25 mg/kg/min adjusted based on blood pressure and heart rate measurements), two baseline blood samples (1 ml) are taken, then 600 mg/kg of glucose is administered as a bolus injection and additional 1 ml blood samples are collected at 2,3,4,5,6,8,10,12,14,16,19,22,23,24, 25,27,30,40,50,60,70,80,90,100, 120,140,160,180 minutes after glucose. Blood sample volumes collected will be within CRPRC guidelines.

Serum Lipids: Samples for serum lipids (5 ml) will be obtained from all animals both in the fasting state and in the postprandial state before and at each timepoint during the dietary intervention period.

Anesthesia: Biopsies and the DEXA scans will be done under the same anesthesia. The other test that requires anesthesia is the Fast Sampled Intravenous Glucose Tolerance Test (FSIVGTT). The most frequent testing period is during the first month. No animal will be anesthetized more frequently than once a week during that first month or at any other time during the study. For months 4 through 60, the average number of procedures requiring anesthesia will be two every three months.

Testing Schedule							
Year 1	Baseline	2 Weeks	1 Month	2 Month	4 Month	6 Month	12 Month
Muscle/fat biopsy	x		x				
DEXA	x		x	x	x	x	x
Total Body Water	x	x	x	x	x	x	x
FSIVGTT	x	x	x	x	x	x	x
Metabolic rate	x	x	x	x	x	x	x
Energy Expenditure	x	x	x	x	x	x	x
24-hour leptin	x	x	x	x	x	x	x
Lipid panel	x	x	x	x	x	x	x
Year 2-5	3 Month	6 Month	9 Month	12 Month			
Muscle/fat biopsy	x						
DEXA	x	x	x	x			
Total Body Water	x	x	x	x			
FSIVGTT	x	x	x	x			
Metabolic rate	x	x	x	x			
Energy Expenditure	x	x	x	x			
24-hour leptin	x	x	x	x			
Lipid panel	x	x	x	x			

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	Control Animals (sucrolose-sweetened solution)	8	2
2	Fructose Fed Animals (500 ml 20% fructose koolaid/day)	8	2
3	Glucose Fed Animals (500 ml 20% glucose koolaid/day)	8	2

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p>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.</p>
2	<p>Minor stress or pain of short duration</p> <p>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</p>
3	<p>Moderate to severe distress</p> <p>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</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p>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.</p>

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?

Nonhuman primates are considered to be more relevant models of human physiology and nutrition than rodents. Spontaneous adult onset obesity in monkeys is likely to be analogous to this type of obesity in humans. Long term studies with fructose feeding have been conducted in rodents but not in humans or nonhuman primates. The number of animals required was estimated by power analysis where the expected proportion of within subject variance accounted for by the experimental treatments was set at 0.3, power=0.8 and p=0.05. Under these criteria, it is estimated that a sample size of 6-8 animals will allow for the detection of differences > 1.64 standard deviations.

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

Building:

CRPRC

Room:

Procedure Room

Who will be the surgeon?

CRPRC Veterinary Staff

A 1.0 g fat biopsy will be obtained from the interscapular region. A 0.5 g muscle biopsy will be obtained from an epaxial muscle in the same region. All procedures will be performed under ketamine anesthesia (10-15 mg/kg IM).

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 Monkey	Ketamine	10-15 mg/kg	IM	Before DEXA Scanning and for FSIVGTT Trials
Rhesus Monkey	Ketamine	0.05- .25 mg/kg/min	IV	During FSIVGTT Trials
Rhesus Monkey	Diazepam	1 mg/kg	IM	Before FSIVGTT Trials

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)

No adverse effects are expected

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.

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:

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

What was the date on which you conducted this search?

8/20/98 9/13/01

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	1966-1998	Monkeys Fructose Feeding
Pub Med	1998-2001	Monkeys Fructose Feeding

What were your findings with respect to alternative methodologies?

No alternative methodologies are available

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?

Animals will be euthanized if they become ill or debilitated such that the veterinary staff of the CRPRC feels that euthanasia is indicated.

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 Monkey	Overdose	Pentobarbital	100 mg/kg	IV

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

They will be returned to the CRPRC colony.

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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