

**PROTOCOL FOR ANIMAL USE AND CARE**Email to: [campusvet@ucdavis.edu](mailto:campusvet@ucdavis.edu)

EH&amp;S USE ONLY

**PROTOCOL: 10289**  
**EXPIRES:**

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

Species (common names):	Number:	Source:
Rhesus Monkeys	20	CRPRC

<b>Project Title</b>	Effects of a modulator of fatty acid metabolism on insulin sensitivity and lipids		
Overnight housing location::	CRPRC	Day use only :	CRPRC
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.

After a 3 week baseline period, 14 obese, insulin resistant, hyperlipidemic monkeys will receive a high or low oral dose, B.I.D., of a modulator of fatty acid metabolism drug provided by Merck Pharmaceutical. Six animals will serve as controls and will receive vehicle only. The vehicle will be either a fruit snack or sweetened solution, whichever the animal prefers. Blood samples will be collected before, during, and 2 weeks post treatment. Ad libitum food intake will be monitored. DEXA scans will be performed to assess body composition. IV glucose tolerance, fatty acid synthesis, and metabolic rate will be determined.

**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.

Throughout the 11 week experiment, ad libitum food intake will be monitored and monkeys will receive morning (8AM) and afternoon (4PM) fruit snacks or sweetened solutions (whichever each monkey prefers). Drug will be administered orally in fruit snacks or as a sweetened solution, B.I.D. Monkeys will be fasted for 16 hours before fasting blood draws and procedures that require anesthesia.

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 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 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:	Merck	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 [pcillman@ucdavis.edu](mailto:pcillman@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.

Compounds that modulate fatty acid metabolism may be useful for treating hyperlipidemia, obesity, and/or insulin resistance. Merck has tested a compound that modulates fatty acid metabolism and found it caused a mechanism-based improvement in lipid profiles in rodents. We propose to examine the effects of this compound developed by Merck on serum lipids, circulating hormones, glucose tolerance, body composition, and metabolic rate in obese and insulin resistant monkeys.

**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 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 Protocol:** The 20 animals (7 low dose drug, 7 high dose drug, 6 control) will be divided into two cohorts of 10 animals each as described:

**Cohort 1**

Drug low dose - 4 animals  
 Drug high dose - 3 animals  
 Vehicle - 3 animals

**Cohort 2**

Drug low dose - 3 animals  
 Drug high dose - 4 animals  
 Vehicle - 3 animals

For each cohort there will be a 3 week baseline, a 6 week drug treatment, and a 2 week post-treatment period totaling 11 weeks.

**General Feeding and Body Weight Protocol:** Throughout the 11 week experiment, animals will be weighed weekly. They will be fed their usual chow diet ad libitum and food intake will be monitored twice daily. The 20 animals selected for this study are obese (BMI  $\geq$  35), insulin resistant and/or hyperlipidemic. All these parameters are positively affected by an energy restricted diet. Ad libitum feeding ensures that none of the animals are in negative energy balance. Therefore any positive results during dosing can be attributed to the drug and not to the effects of energy restriction.

**Drug Administration:** Animals will receive fruit snacks or non-nutritive sweetened solution 2 times/day throughout the 11 week experiment. During the treatment period, drugs will be administered B.I.D. orally (1mg/kg 2Xs/day low dose and 10 mg/kg 2Xs/day high dose) inside the fruit snack or dissolved in the sucrose- sweetened solution. The daily dose of drug for each animal will be weighed and placed inside a fruit snack (small banana or apple) and handed to the animal. If an animal does not consistently consume his fruit snack with drug, the drug will be provided in a sweetened solution to drink. The animal technician will monitor each animal's preference and provide the drug in the vehicle most likely to ensure a consistent drug intake.

**Metabolic Rate (MR):** During Weeks 1 and 7, animals will be transported 2 at a time to the CRPRC Exposure Facility for 24 hours metabolic rate measurements. The MR system consists of 2 MR chambers which contain 32½X26X40" primate cages, an O<sub>2</sub> analyser and a CO<sub>2</sub> analyser. A flow controller and channelyser allows for air flow rate adjustments and for sequential channeling of the air flow from the air reference line and the two animals chambers through the CO<sub>2</sub> and O<sub>2</sub> analyzer. The system measures and reports O<sub>2</sub> consumption, CO<sub>2</sub> production, respiratory quotient (RQ), and heat production. Animals are provided with water bottles and ad libitum chow during the 24 hour monitoring period. Animals will be acclimated to the chambers on at least 2 separate occasions before their first 24-hr MR trial.

**Fasting Hormone and Lipid Blood Collection:** Six times, during Weeks 1,2,5,7,9 and 11, eight ml of blood will be collected for fasting serum lipids, chemistry and CBC analysis. Another seven ml of blood will be collected in EDTA for fasting glucose, insulin, leptin, adiponectin, glucagon, free fatty acids analysis. Total blood collected at each of these six collection times is 15 mls. Blood sampling volumes will conform to CRPRC guidelines.

**Intravenous Glucose Tolerance Test (IVGTT):** Two times, during Weeks 3 and 9 IVGTTs will be performed. Monkeys will be fasted at 4 PM on the prior day. On the study day, one 3ml blood sample will be collected at 7:00AM. Animal will then receive an initial dose of ketamine (15 mg/kg, i.m.) followed after 20 minutes by diazepam (Valium, 1 mg/kg, i.m.). IV catheters will be inserted into two arm or leg veins. The CRPRC SRA/animal technician will insert the catheters into whichever of the arm or leg veins they determine to be most easily catheterized. Supplemental ketamine will be given as an i.v. infusion at a rate of 0.25-1 mg/kg/min (0.125 ml/kg/min of a solution containing 2 mg/ml) and the infusion rate will be adjusted to maintain a stable plane of anesthesia as assessed by monitoring of blood pressure, heart rate, respiratory rate and muscular rigidity. Three baseline samples (3 ml) will be collected from one catheter at -10, -5 and 0 minutes. Then 600 mg/kg of 50% dextrose

is administered in the contralateral catheter. Additional 3 ml blood samples are collected at 1, 3, 5, 10, 15, 20, 30, 40, and 60 minutes. All blood sample volumes collected will conform to CRPRC guidelines.

**Body Composition:** Two times, during Weeks 3 and 8, percent and total body fat will be determined under ketamine-medetomidine anesthesia IP(10-20mg/kg and 30 ug/kg respectively) by dual energy X-ray absorptiometry (DEXA) scan with the DEXA unit located in TB 175. Animals will be transported to TB 175 in a CRPRC van in animal transport cages. All handling of the animals, including transport, injections and positioning for the scan, will be done by CRPRC technicians. Each animal is anesthetized and then scanned 2 times for approximately 10-15 minutes/scan. DEXAs will be performed at least 2 days before or 2 days after the IVGTT (also scheduled for Week 3) and the fasting blood draw (also scheduled for Week 8).

**Assessment of Fatty Acid Synthesis:** Two times, during Weeks 2 and 8, fatty acid synthesis will be assessed by measuring the enrichment of deuterium into fatty acids. At 6AM, the day of procedure, the animals' food will be removed and they will be fasted for the next 10 hours. At 8AM, a 4 ml blood sample will be collected, followed by a 50ml subcutaneous injection of D2O. 4 ml blood samples will be collected at 2,4,6 and 8 hours post-injection. Animals will be fed after the 8-hour post-injection blood draw.

**Blood Collection for Drug Pharmacokinetics (PK) Analysis:** On the first day and final day of drug treatment, 3 ml blood samples will be collected for drug PK analysis. Blood will be collected at 0, 0.5, 1, 2, 4, 8, and 24 hours after dosing.

### **Schedule for blood draws, metabolic rate, and body composition procedures**

<b><u>Timeline (in weeks)</u></b>	<b><u>PROCEDURE</u></b>
1 Baseline	Metabolic Rate, Blood collection for hormones/lipids
2 Baseline	Blood collection for hormones/lipids, Fatty acid synthesis assessment
3 Baseline	IVGTT, DEXA (a minimum of 48 hours between procedures)
4 Treatment	Blood collection for pK drug analysis
5 Treatment	Blood collection for hormones/lipids
6 Treatment	
7 Treatment	Metabolic rate, Blood collection for hormones/lipids
8 Treatment	DEXA, Fatty acid synthesis assessment
9 Treatment	Blood collection for hormones/lipids, IVGTT, Blood collection for PK anal.
10 Post-treatment	
11 Post-treatment	Blood collection for hormone/lipid

**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.

<b>Group</b>	<b>Procedures / Drugs</b>	<b>Number of Animals</b>	<b>Category</b>
1	Drug - FA metabolism modulator - Low dose	7	2
2	Drug - FA metabolism modulator - High dose	7	2
3	Vehicle	6	2

## Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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?

Nonhuman primates are considered to be more relevant models of human physiology and nutrition than rodents. Based on the previous study we have done for Merck testing a PPAR compound (protocol #9642), Merck has determined that 7 animals in each drug group are required to detect changes of 15% compared to the control group.

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 Veterinarian

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	4 times: Injection prior to IVGTT, DEXA scans and CT scans
"	Medetomidine	20-40 µg/kg	IM	Better relaxant than ketamine alone for DEXA
"	Atipamazole	20-40 ug/kg	IM	Medetomidine Reversal
Rhesus monkey	Diazepam	1 mg/kg	IM	Before FSIVGTT trials
Rhesus monkey	Ketamine	0.05-.25 mg/kg/min	IV	Maintenance Infusion During FSIVGTT trial

**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)

In previous studies conducted by Merck with this modulator of fatty acid metabolism, no adverse effects have been observed in rodents or in monkeys at these doses.

No adverse effects resulting from the procedures are anticipated beyond those associated with blood sampling such as bruising at the site of blood collection or injection/catheter placement sites.

It is possible the ad libitum feeding may cause some of the animals to attain body weights greater than 15 Kg. The upper weight specification for their 6.2 cubic foot cages is 15 Kg.

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.

Food intake will be assessed daily, body weight weekly, and CBC and serum chemistry analysis will be performed every three weeks during the trial. These measurements will allow us to determine if animals are experiencing any adverse effects of the drug. If an animal decreases its food intake by more than 50% during any week, loses more than 30% of body weight (the animals are currently obese), or shows clinically relevant adverse changes in serum chemistry/hematology parameters, it may be removed from the trial at the discretion of the CRPRC Veterinary Staff in consultation with the Principal Investigator.

The discomfort associated with the procedures should be temporary and minimal. Analgesics can be provided at the discretion of the CRPRC Veterinary Staff.

We do not expect that animals that attain a BW above 15 Kg will experience discomfort in the standard size cages. The additional weight gain caused by ad libitum feeding will increase body fat, but not increase body frame size. It may also cause the animal to become less active. Therefore the 6.2 cubic foot cage should still be adequate for the animals that gain weight.

*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/Databases\\_Med\\_Vet\\_Researchers.htm](http://trc.ucdavis.edu/jawelsh/Databases/Databases_Med_Vet_Researchers.htm) (email: [jawelsh@ucdavis.edu](mailto:jawelsh@ucdavis.edu))*

*or [http://www.vetmed.ucdavis.edu/Animal\\_Alternatives/main.htm](http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm) (email: [mwwood@ucdavis.edu](mailto:mwwood@ucdavis.edu))*

What was the date on which you conducted this search?

6-20-02

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
PubMed	1965-2002	"modulator of fatty acid metabolism," obesity, adipose distribution, lipid profile, insulin resistance, monkeys
ISI Web-of-Science	1975-2002	"modulator of fatty acid metabolism," obesity, adipose distribution, lipid profile, insulin resistance, monkeys

What were your findings with respect to alternative methodologies?

There are no alternative methodologies to examine the effects of these compounds in monkeys.

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.

The study has not been previously conducted.

**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	60 mg/kg	IV

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

All animals will be returned to colony.





