The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) has adopted the following guideline to clarify the responsibilities of the institution, the IACUC, Attending Veterinarian, and research personnel and to outline the minimally acceptable standards for regulating food and fluids in nonhuman primates. Food and fluid regulation are sometimes necessary in studies involving physiological, neuroscience and behavioral research protocols. This guideline applies to researchers using non human primates.

*Guide for the Care and Use of Laboratory Animals* (2011) Food and Fluid Regulation pgs. 30-31

“The objective when these studies are being planned and executed should be to use the least restriction necessary to achieve the scientific objective while maintaining animal well-being.”

**Definitions Fluid Regulation**

**Average daily fluid consumption:** The average amount of water that an animal consumes per day of *ad libitum* water during a two week period. This is necessary when a percentage of *ad libitum* water consumption is used to calculate the amount of fluid given during regulation.

**Fluid regulation:** The act of controlling the amount of water an animal receives and when the animal receives it. The amount is less than the average daily water consumption. An animal that is offered less than 80 ml/kg per day is considered to be regulated (1).

**Fluid ration:** The total volume of fluid (i.e. water or juice) allotted to the animal per day, both during and outside of the testing time frame.

**Baseline weight fluid regulation:** Determined during the initial fluid regulation process. For fluid regulation in naïve animals, each decrease in the amount of water lasts for one week, with the animal weighed daily. The regulation process begins when water is regulated to 80ml/kg. Weights are submitted to the veterinarian for approval to restrict to 60ml/kg, 40ml/kg and 20 ml/kg. The baseline weight is the average weight at 40ml/kg.

**Fluid Regulation**

1. Animals should be given the largest amount of fluid possible to achieve research goals. Fluid regulation must be customized to the individual animal.
2. Prior to the implementation of fluid regulation, the animal will undergo a health assessment by ULAR veterinary staff. If the animal is deemed healthy, the fluid regulation process may begin.
3. Baseline weight will be determined during the initial fluid regulation process. The baseline weight should be reassessed quarterly in growing animals. The baseline weight in all other animals, will be reassessed based on recommendations by the ULAR veterinary staff.
4. Changes in the amount of administered fluids should be done gradually over the course of a week at a time, plus or minus 20 ml/kg/day, to avoid physiological stress caused by acute dehydration.
5. Each animal must be observed daily during fluid regulation. Food intake, consistency of stool, amount of urine and behavior of the animal should be recorded daily.
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6. Animals on fluid regulation must be weighed at least once a week. The IACUC does not recommend the use of immature animals (less than 5 years of age) for fluid regulation protocol. If immature animals must be used on fluid regulation protocols, they must be weighed three times a week.

7. Weight loss of more than 10% of baseline weight must be reported to the ULAR veterinary staff.

8. Signs of dehydration such as decreased food intake, little or no urine production, scant hard dry feces, lethargy, sunken eyes, increased skin turgor or behavioral changes must be reported to the ULAR veterinary staff.

9. If the daily minimum amount of fluid is not provided during the session, additional fluids should be given to meet the minimum requirements. Each animal should be given the opportunity to earn fluids to satiety during each session.

10. Animals should not go below 20 ml/kg/day of fluid. This is an estimate of fluid loss related to daily biological processes (respiration, urination, defecation, etc) for nonhuman primates.(2)

11. If it is necessary to go below 20 ml/kg/day, the ULAR veterinary staff must be notified and increased monitoring of that animal will be done by the lab staff, ULAR veterinary staff and animal caretakers. Veterinary assessments may include, but are not limited to, urine specific gravity, daily weight assessment and blood testing.

12. Records for fluid regulated animals must be maintained daily. The records must be available for evaluation by ULAR veterinarians, IACUC, ORA compliance staff or federal regulatory representatives.

13. Animals anesthetized for scheduled procedures should be removed from water regulation at least 24 hours prior to the procedure, in consultation with a ULAR veterinarian. For major surgical procedures, the animals should remain off of water regulation for a minimum of one week post procedure. Transition back to water regulation must be done gradually with no more than a 20 ml/kg/day decrease.

Definitions Food Regulation

Food regulation: The act of controlling the amount of food an animal receives and when it receives it. The amount is less than the NRC 100% ration. It has been shown that maintenance of weekly chow intake in the range of 70–85% of NRC ration recommendations for metabolizable energy is necessary for consistent behavioral responding. (3)

National Research Council (NRC) recommended daily ration level: NRC recommended number of biscuits per day using the equation: \( Y = 16.504 X ^{-0.8219} \) where \( Y \) is the number of biscuits per kilogram per day and \( X \) is the animal’s body weight in kilograms. (3) See chart for number of biscuits.

NRC 100% ration: Once the number of daily biscuits is determined, that number, the NRC 100% ration, should be fed for two weeks. The NRC 100% ration should be split into 2 meals a day.

NRC 85% ration: This is the recommended starting ration for regulation.

Baseline weight food regulation: Determined prior to beginning the food regulation process.

Food Regulation

1. Animals should be given the largest amount of food possible to achieve research goals. Not all animals will perform well with food regulation as a behavioral motivator.
2. Food regulation must be customized to the individual animal. Mature or obese animals are more tolerant to greater restriction than young or thin animals.

3. Prior to the implementation of food regulation, the animal will undergo a health assessment by ULAR veterinary staff. If the animal is deemed healthy, the food regulation process may begin.

4. After 2 weeks of 100% NRC ration is fed, the animal maybe reduced down to 85% of NRC ration. If more regulation of food is necessary due to poor performance, regulation may go down to 70% of NRC ration. Regulation below 70% of NRC ration needs to be approved by the veterinary staff.

5. Animals must be observed daily while they are on food regulation. Food intake, consistency of stool, amount of urine and behavior of the animal should be recorded daily.

6. Food regulation should be introduced gradually, such as 10 to 15% regulation per week.

7. Baseline weight and body condition score, on a 5 scale; will be determined prior to the initial food regulation process. The baseline weight should be reassessed quarterly in growing animals. Baseline weight reassessment in all other animals will be done based on recommendations by the ULAR veterinary staff.

8. Animals on food regulation must be weighed at least once a week and have a body condition score at least once a month. Immature animals must be weighed three times a week and a body condition score must be assessed weekly.

9. Animal’s weight will fluctuate throughout the day. It is important to weigh the animal at the same time each day and prior to receiving food.

10. Weight changes of more than 15% of baseline weight must be reported to the ULAR veterinary staff. Body condition score less than 2.0 must be reported to the ULAR veterinary staff.

11. Daily records for animals on food regulation must be maintained. The records must include amount of food provided, amount of food consumed at each meal, percent of restriction from 100% NRC ration, weight, body condition score, and performance on behavioral testing. The records must be available for evaluation by ULAR veterinarians, IACUC, ORA compliance staff or federal regulatory representatives.

12. On non-testing days, such as the weekends, the animal should receive a 100% NRC ration divided into two equal meals about 6 hours apart. This can reduce the risk of bloat.

Procedures for Protocol Submission

1. Justification for performing procedure. Scientific justification must be provided for using fluid or food regulation, including:
   a. why the regulation must be undertaken
   b. what research information the regulation is designed to address
   c. the level or ranges to which food or fluid will be regulated
   d. the length of time of the regulation is required

2. Literature search for alternatives. An extensive and complete alternatives literature search must be performed by the PI and approved by the IACUC. Appropriate keywords for the search include, but are not limited to: food restriction, fluid and water restriction, food deprivation, fluid and water deprivation, positive reinforcement and “alternative”.
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These terms should be combined with specific keywords related to the animal model and type of study.

3. **Description of how the level of regulation will be determined for each individual animal.**
   As per the *Guide*, the level of regulation is typically measured as a percentage of the *ad libitum* daily intake or as a percentage change in an animal’s body weight.

4. **Description of the experimental conduct of the regulation.** The procedures for training and maintaining food or fluid regulation in the nonhuman primates must be clearly described in the IACUC protocol, including:
   a. the plan for gradual incremental reductions for food/fluid
   b. length of experimental time the animals’ food/fluid will be scheduled (days, week, months)
   c. frequency at which the animals’ food/fluid will be scheduled (e.g. “everyday”, “every other day”, “X days a week”, etc.)
   d. criteria for removal or the thresholds of clinical signs and other parameters that may necessitate the animal being removed from the experimental testing procedures.
      Please consult with ULAR veterinary staff to best determine humane parameters.

5. **Description of monitoring the animals.** Describe who will be monitoring the animals during the course of the regulation, how often they will be observed, how often they will be weighed, the parameters that will be used to monitor their health, and criteria used to determine whether an animal should be removed from the study.

Exemptions to animal welfare regulations

**Animal Welfare Regulations 3.82 (a-b)**
[Food]...must be of sufficient quantity and have sufficient nutritive value to maintain a healthful condition and weight range of the animal and to meet its normal daily nutritional requirements. Nonhuman primates must be fed at least once each day except as otherwise might be required to provide adequate veterinary care.

**Animal Welfare Regulations 3.83**
Potable water must be provided in sufficient quantity to every nonhuman primate housed at the facility. If potable water is not continually available to the nonhuman primates, it must be offered to them as often as necessary to ensure their health and well-being, but no less than twice daily for at least 1 hour each time, unless otherwise required by the attending veterinarian, or as required by the research proposal approved by the Committee at research facilities.

If food (at least once daily in sufficient quantities) or water (offered *ad libitum*, at least twice daily for 1 hour periods) cannot be made available to nonhuman primates due to the requirements of the research model, then an exemption form must be submitted by the PI and approved by the IACUC prior to the initiation of any food/fluid regulation. For ARIES protocols, the exemption must be completed via ARIES.

At any time, ULAR veterinary staff has the authority to provide nutritional or fluid support to any nonhuman primate, on- or off-study, to maintain the health and well-being of the animal.
Cited References


General References

Association of Primate Veterinarians Food Restriction Guidelines for Nonhuman Primates in Biomedical Research.


The Guide for the Care and Use of Laboratory Animals, National Research Council, 1996.

The Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.


Guidelines for Diet Control in Animal Studies, National Institutes of Health (NIH).

Willems, RA. 2009. Regulatory issues regarding the use of food and water restriction in laboratory animals. Lab Animal 38(10).
### Body Condition Scoring of Nonhuman Primates Using
Macaca mulatta as a Model

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>EMACIATED</strong> — Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal or no muscle mass is palpable over ilium or ischium. Anus may be recessed between ischial calliities. Body is very angular, no subcutaneous fat layer to smooth out prominences.</td>
</tr>
<tr>
<td>1.5</td>
<td><strong>VERY THIN</strong> — Hips, spinous processes, and ribs are prominent. Facial bones may be prominent. There is very little muscle present over the hips and back. Anus may be recessed between ischial calliities. Body is angular, no subcutaneous fat to smooth out prominences.</td>
</tr>
<tr>
<td>2</td>
<td><strong>THIN</strong> — Very minimal fat reserves, prominent hip bones and spinous processes. Hips, spinous processes and ribs are easily palpable with only a small amount of muscle mass over hips and lumbar region.</td>
</tr>
<tr>
<td>2.5</td>
<td><strong>LEAN</strong> — Overlying muscle gives hips and spine a more firm feel. Hip bones and spinous processes are readily palpable, but not prominent. Body is less angular because there is a thin layer of subcutaneous fat.</td>
</tr>
<tr>
<td>3</td>
<td><strong>OPTIMUM</strong> — Hip bones, ribs and spinous processes are palpable with gentle pressure but generally not visible. Well developed muscle mass and subcutaneous fat layer gives spine and hips smooth but firm feel. No abdominal, axillary or inguinal fat pads.</td>
</tr>
<tr>
<td>3.5</td>
<td><strong>SLIGHTLY OVERWEIGHT</strong> — Hip bones and spinous processes palpable with firm pressure but are not visible. Bony prominences smooth. Rib contours are smooth and only palpable with firm pressure. Small abdominal fat pad may be present.</td>
</tr>
<tr>
<td>4</td>
<td><strong>HEAVY</strong> — Bony contours are smooth and less well defined. Hip bones, spinous processes and ribs may be difficult to palpate due to more abundant subcutaneous fat layer. May have fat deposits starting to accumulate in the axillary, inguinal or abdominal areas.</td>
</tr>
<tr>
<td>4.5</td>
<td><strong>OBESE</strong> — This animal will often have prominent fat pads in the inguinal, axillary or abdominal region. Abdomen will be pendulous when animal sitting or ambulating. Hip bones and spinous processes difficult to palpate. Bony contours smooth and poorly defined.</td>
</tr>
<tr>
<td>5</td>
<td><strong>GROSSLY OBESE</strong> — Obvious, large fat deposits in the abdominal, inguinal and axillary regions. Abdominal palpation is very difficult; due to large amount of mesenteric fat. Pronounced fat deposits may alter posture/ambulation. Hip bones, rib contours and spinous processes only palpable with deep palpation.</td>
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