



Food and/or Fluid Regulation

352.1 Purpose

This policy establishes standards and expectations for researchers performing food or fluid regulation in healthy mice, rats and nonhuman primates (NHP) for experimental purposes. The principles in this policy can also be used as a basis for decisions regarding other species or animals with physiologic impairment, with relevant alterations included in the IACUC protocol. This policy does not apply to animals restricted at the advice of the veterinary staff or in preparation for anesthesia.

352.2 Definitions

- **Regulation** is a deviation from the standard husbandry practices in the amount or availability of food or water. It includes scheduling and restriction as defined below. Special diets are not inherently considered regulation.
- **Scheduling** of access to food or fluid limits the number of times or a length of periods during which the animal has access to food or fluid so that the animal consumes a normal portion but at intervals or durations that differ from standard husbandry practices (ILAR, 2003). This definition only applies if food or fluid is removed for a period of greater than 12 hours. Scheduled feeding is not expected to result in a subnormal body weight.
- **Restriction** is the provision of rations such that the volume of food or fluid is strictly monitored and controlled (ILAR, 2003). Restricted feeding typically limits the total volume of food or fluid consumed for the purpose of reducing the animal's weight to a level lower than that expected for an *ad libitum* fed animal.
- **Ad libitum** food intake is the amount of food consumed when the animal has free access to food at all times.

352.3 Protocol Considerations

352.3.1 Scientific Justification

Food or fluid regulation may be required for the conduct of some physiological, neuroscience and behavioral research protocols. The type and extent of regulation must be described and justified in the approved IACUC protocol that covers the animal(s) in question (ILAR, 2011; AWR). Researchers must state in the protocol the necessary level of regulation, potential adverse consequences of regulation, and methods for assessing the health and well-being of the animals (ILAR, 2011; AWR). The following refinements and alternatives should be considered, if applicable to the particular reason for regulation:

352.3.1.1 The least restrictive schedule that will achieve scientific objectives should be utilized (ILAR, 2011).

352.3.1.2 Use of a highly preferred food or fluid as positive reinforcement either employed instead of restriction, or to maintain behaviors once the animal is trained (ILAR, 2011)

352.3.1.3 Use of preference tests to choose the primary reinforcer may increase motivation and improve training outcome (Westlund, 2011).

352.3.1.4 Consideration of opportunities for breaks from the restriction schedule or ease of the regulation once the animal is trained. For example, attempts could be made in trained animals to increase reward amounts to boost total food or fluid intake, consistent with achieving the required number of behavioral trials (Toth, 2000).

352.3.1.5 Consideration of subject suitability towards training before and during the period of training. Researchers should follow clear rules to determine whether the training is effective, and whether it can be done without excessive negative behavioral consequences (eg. stereotypies, increased aggression, etc.).

352.3.1.6 Researchers should work with the Behavior Management Unit at the Yerkes Center if using NHP to refine their training methods (for instance, by establishing conditioned reinforcers or using shaping techniques).

352.3.2 Pain & distress classification: IACUC must consider if food or fluid restriction causes distress which warrants Class E status (Willems, 2009; APHIS AC, 2011). Alteration of a normal food or fluid schedule is not inherently associated with unrelieved pain or distress, although food restriction can be associated with chronic stress in both humans and animals (Heiderstadt, 2000; Marinelli, 1996; Tomiyama, 2010). Animals can therefore be categorized as stress level Class C if no adverse consequences are expected. Class E is recommended if the goal is to maintain animals at less than or equal to 85% of expected weight.

352.3.3 Implementing regulation

Restrictions must be conducted with care and tailored to the feeding patterns and nutritional requirements of the animals' age, health status, strain and species as well as the requirements of the study. The approved IACUC protocol must include the following details. Additional species specific considerations are below (See 352.5).

- Type of ration which will be provided: The type of food or fluid.
- Time period of required regulation
- Personnel responsible for providing the diet.
- Amount of ration which will be provided daily: Described as either weight of food or volume of fluid and whether provided as restricted for scheduled feeding. A percent *ad libitum* food or fluid intake can also be used if the method for determining this amount is clearly described. For food regulation, this should include a target end weight for the animal (eg. 90% of expected weight). The

method for determining the animal's expected weight should be included (i.e. comparison with baseline, age-matched controls or growth chart).

352.3.4 Potential Adverse Consequences

The potential consequences and related animal welfare concerns of regulation should be described within the approved IACUC protocol. Both intentional (e.g. weight loss) and unintentional (e.g. dehydration) consequences should be considered. Intervention criteria and endpoints based on these consequences must be defined in the protocol. Intervention may include the provision of the restricted item (food or water) and treatment for dehydration. If animals reach intervention criteria, the veterinary staff should be notified and the animal removed from regulation until the clinical signs resolve. Animals can be returned to study if interventions are effective in returning animal to normal behavior, normal hydration status and the animal's weight is at or above the target weight. Animals which fall into intervention criteria more than one time or cannot be returned to target levels within 48 hours (water deprivation) or 3 days (food deprivation) must be removed from study or have IACUC approval for additional use. Animals cannot be returned to study until they have met these guidelines for at least 24 hours. The veterinary staff may request that animals are temporarily or permanently removed from study based on other IACUC endpoints.

352.3.5 Health Assessments

Personnel involved in food and fluid restriction protocols should be able to recognize signs of distress, hydration status and body condition score in the animals. The protocol must include a schedule for monitoring the following health parameters. This monitoring schedule is based on federal regulatory requirements (ILAR 2011, AWR).

- Baseline (obtained within the week before starting regulation)
 - Body weight
NHP body weights should be taken at a consistent time of day and prior to feeding and watering
 - Body condition score (Appendix A, B)
 - Behavior (attitude, activity, abnormal behaviors)
- Once weekly
 - Body weight
 - Body condition score
- Daily
 - Hydration status
 - Behavior and clinical changes
 - Singly housed animals: presence urine and feces
 - Any other criteria if used as parameter for intervention or removal from study
 - Amount of food or fluid provided and consumed

352.3.6 Documentation (Appendix C)

Documentation of providing provisions and health assessments must be maintained by the research staff and easily accessible for review either in the housing room or other location as designated by the veterinary staff and animal care. An example form is available (Appendix C), but may be revised in consultation with the veterinary and animal care staff.

352.4 Animal Identification and Signage (Appendix D).

Animals that are on food and water restrictions will be housed in clearly marked cages, runs, or rooms. Signage information should include emergency contact information, typical procedures for feeding the animals and procedures for which animal care will provide food if animal care cannot verify that the animals have been fed by the lab (Appendix D).

352.5 Species Specific Considerations

352.5.1 Rats & mice

352.5.1.1 Age

- Food or fluid regulation is not recommended in rodents < 14 weeks old

352.5.1.2 Food restriction (ILAR, 2003)

- Full grown animals can be fed 70% of *ad libitum* food consumption until they reach 90% of a baseline weight. *Ad libitum* food consumption can be determined by weighing the food daily or based on an established precedent for the given age and species of animal. Volumes as low as 50% of *ad libitum* food consumption for a target weight of 80% of baseline may be scientifically justified in some circumstances.
- The food must be gradually reduced to the target percent of *ad libitum* over at least one week period, and longer periods of up to several weeks are associated with less of a stress response (ILAR, 2003).
- Fasting for 24 hours or more is associated with stress and should be avoided unless scientifically justified (Nowland, 2011).

352.5.1.3 Fluid regulation

- Scheduled fluid regulation should make water available for at least 15 minutes a day (Heiderstadt, 2000).
- Restricted fluid regulation usually involves a percentage of *ad libitum* fluid intake that is permitted outside of the testing sessions, and can vary widely depending on the species and task (ILAR, 2003).
- Food must be provided at the same time as water as rodents often will not eat if water is not available.

352.5.2 NHP

- Animals may not be both food and water restricted.

- NHP's on water restriction are provided free water two days per seven day week. During the five working days, fluid is given as part of the testing procedure to provide reinforcement to perform motor or cognitive tasks.
- NHP's may be food restricted overnight or 90% of *ad libitum* fed body weight, while allowing compensation over time for growth, as a task motivator. Food restriction for the entire length of the study is not to exceed 10% body weight loss.
- The amount of food ration that any animal can be restricted to safely and humanely will vary by individual animal. Unless empirical data are established, no less than 85% of the National Research Council determined full ration should be fed³.
- Food restriction should be introduced gradually, such as 5% per month, if the individual animal is not performing as expected. It should be noted that not all animals will perform well with food restriction as a behavioral motivator.³ Acclimating animals to their new feeding schedule over time may also mitigate any potential stress response.

352.6 References

1. Animal and Plant Health Inspection Service Animal Care (APHIS AC). 2011. Policy #11: Painful and Distressful Procedures.
2. Animal Welfare Act Regulations. (n.d.). 9 CFR. Chapter 1. Subchapter A. Part 2.38 (f) (2) (ii)
3. Association of Primate Veterinarians. NHP Food Restriction Guidelines.
4. Institute for Laboratory Animal Research. (2003). *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*. Washington, D.C. : The National Academies Press.
5. Institute for Laboratory Animal Research. (2011). *The Guide for the Care and Use of Laboratory Animals*. Washington, D.C. : The National Academies Press.
6. Heiderstadt KM, McLaughlin RM, Wright DC, Walker SE, Gomez-Sanchez CE. 2000. The effect of chronic food and water restriction on open-field behaviour and serum corticosterone in rats. *Laboratory Animals* 34: 20-28.
7. Marinelli M, Le Moal M, Piazza PV. 1996. Acute pharmacological blockade of corticosterone secretion reverses food restriction-induced sensitization of the locomotor response to cocaine. *724*: 251-5.
8. Nowland MH, Hugunin KMS, Rogers KL. 2011. Effects of Short-Term Fasting in Male Sprague–Dawley Rats. *Comp Med* 61: 138-144.
9. Prescott MJ, Brown VJ, Flecknell PA, Gaffan D, Garrod K, Lemon RN, Parker AJ, Ryder K, Schultz W, Scott L, Watson J, Whitfield L. 2010. Refinement of the use of food and fluid control as motivational tools for macaques used in behavioral neuroscience research: Report of a Working Group of the NC3Rs. *Journal of Neuroscience Methods* 193: 167-188.
10. Rowland NE. 2007. Food or Fluid Restriction in Common Laboratory Animals: Balancing Welfare Considerations with Scientific Inquiry. *Journal of Comparative Medicine* 57: 149-160.
11. Toth LA, Gardiner TW. 2000. Food and Water Restriction Protocols: Physiological and Behavioral Considerations. *Contemporary Topics (by AALAS)* 39, 9-17.
12. Tomiyama AJ, Mann T, Vinas D, Hunger JM, Dejager J, Taylor SE. Low calorie dieting increases cortisol. *Psychosom Med* 72: 357-64.
13. Westlund K. 2011. Can conditioned reinforcers and Variable-Ratio Schedules make food- and fluid control redundant? A comment on the NC3Rs Working Group's report. *Journal of Neuroscience Methods* 204; 202-205.

14. Willems RA. 2009. Regulatory issues regarding the use of food and water restriction in laboratory animals. *Lab Animal* 38, 325-328.

352.7 Property Documents

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Administering Division/Department: IACUC Office

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Appendix A:

**Body Condition Scoring of Nonhuman Primates Using
Macaca mulatta as a Model**

		Ambulating	Right Lateral Viewed from Back
1	EMACIATED – Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal to no muscle mass is palpable over ileum or ischium. Anus may be recessed between ischial callosities. Body is very angular, no subcutaneous fat layer to smooth out prominences.		
1.5	VERY THIN – 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 callosities. Body is angular, no subcutaneous fat to smooth out prominences.		
2	THIN – 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.		
2.5	LEAN – 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.		
3	OPTIMUM – 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.		
3.5	SLIGHTLY OVERWEIGHT – 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.		
4	HEAVY – 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.		
4.5	OBESE – 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.		
5	GROSSLY OBESE – 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.		

Appendix B

	<p>BC 1</p> <p>Mouse is emaciated.</p> <ul style="list-style-type: none"> • Skeletal structure extremely prominent; little or no flesh cover. • Vertebrae distinctly segmented.
	<p>BC 2</p> <p>Mouse is underconditioned.</p> <ul style="list-style-type: none"> • Segmentation of vertebral column evident. • Dorsal pelvic bones are readily palpable.
	<p>BC 3</p> <p>Mouse is well-conditioned.</p> <ul style="list-style-type: none"> • Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.
	<p>BC 4</p> <p>Mouse is overconditioned.</p> <ul style="list-style-type: none"> • Spine is a continuous column. • Vertebrae palpable only with firm pressure.
	<p>BC 5</p> <p>Mouse is obese.</p> <ul style="list-style-type: none"> • Mouse is smooth and bulky. • Bone structure disappears under flesh and subcutaneous fat.

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

Ulman-Cullere M, Foltz C, 1999 Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice, LAS Vol 49 no 3 pg 319-323.



BC 1

Rat is emaciated

- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.



BC 2

Rat is under conditioned

- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.



BC 3

Rat is well-conditioned

- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.



BC 4

Rat is overconditioned

- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.



BC 5

Rat is obese

- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

Hickman D, Swan M, 2010 Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease, JAALAS Vol 49 No 2 pg 155-159.

Appendix D

Food or Fluid Regulation Room Sign (Example)

Animals must be provided their daily rations by (time agreed upon by the laboratory and animal resources program). If the daily documentation for food or water provisions have not been completed by this time, animal care will attempt to contact the contacts listed on this sheet. If they are unable to reach a person within 30 minutes, animal care will provide the standard rations available as part of routine husbandry.

Type of regulation: Food Water Both Protocol #: _____

PI: _____

Time by which rations are provided by the lab:

Animal IDs:

Emergency Contact Information

Primary contact name: _____

Phone 1: _____ Phone 2: _____

Secondary contact name: _____

Phone 1: _____ Phone 2: _____