Division of Research



SUBJECT:	Effective Date:	Policy Nu	ımber:
Diet and Fluid Modifications in Rodents	5/26/2023	10.4.12	
	Supersedes:	Page	Of
	5/22/2020	1	6
	5/26/2017		
	Responsible Authorities:		
	Principal Investigator		
	Vice President, Research		
	Institutional Animal Care and Use Committee		
	Assistant Vice President for Research, Research Integrity		
	Assistant Vice Preside Comparative Medicine		arch,

I. Background

This policy is based on and consistent with the recommendations articulated in (a) the Guide for the Care and Use of Laboratory Animals, 8th ed. (2011), (b) Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research, National Academy of Science (2003) and (c) the Animal Welfare Act and the Animal Welfare Regulations (9 CFR, 1-1-16 Edition). The AWR states that "deprivation of food or water shall not be used to train, work, or otherwise handle animals" unless "short-term withholding of food or water... is specified in an IACUC approved activity that includes a description of monitoring procedures".

II. Purpose

Florida Atlantic University's Institutional Animal Care and Use Committee (IACUC) has adopted the following policy to clarify the scenarios and procedures of regulating food and/or fluid in rodents. Food and/or fluid regulation is typically used in the research setting in (1) studies that use food/fluid consumption to motivate animals to perform novel or learned tasks, (2) studies of the motivated behaviors and physiologic mediators of hunger and thirst, and (3) homeostatic regulation of energy metabolism or food balance. This guideline will outline important issues to be considered during performance of these studies and addressed in protocols that require food and/or fluid regulation/scheduling.

III. General Statement

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All studies proposing to use food or fluid modifications in animals as part of their research must adhere to the procedures described in this policy in order to promote the ethical use of animals in research, teaching or testing.

IV. Policy

- A. Studies proposing to use food or fluid regulation/modifications should be planned to use the least regulation necessary to achieve the scientific objectives while maintaining animal well-being. In order to accomplish this objective, the following must always be considered and adequately described in the IACUC protocol:
 - a. The necessary duration and percentage of restriction compared to ad libitum food and water intake
 - b. Potential adverse consequences of regulation
 - c. Methods to assess the health and well-being of the animals under regulation
- B. Subject suitability towards training before and during the period of food/water regulation must be considered. Researchers should follow clear objectives to determine whether the training is effective, and whether it can be done without excessive negative behavioral and physical consequences (e.g. increased aggressiveness, stereotypies, hunched posture/ruffled fur, chromodacryorrhea).
- C. Ad libitum values need to be determined prior to initiation of food/fluid modification for studies requiring chronic regulation. Published values for the same age, sex, background strain, and weight may be used (See Reference 1 for a sample of published data regarding common strains of mice used in research for water and food values). Rodent vendors often can provide growth curves and food/water intake curves for specific strains as well.
- D. Rodents, especially rats are continuously growing independent of age. That needs to be taken into account when creating a food restriction plan. The absolute food amount provided must be adjusted accordingly over the duration of restriction.
- E. Circadian rhythm of feeding should be considered, especially for rats.
- F. Level of Regulation must be determined prior to initiation of food/fluid restriction and described in the IACUC protocol.
- G. Diet has to meet the animal's nutritional needs unless otherwise scientifically justified. Caloric restriction must not produce unintended nutritional imbalances.
- H. Chronic food restriction can be associated with stress in both humans and animals. Consideration should be given toward pain/distress and how it can be alleviated. Single housing and feeding during the light cycle might aggravate intolerance of food restriction.
- I. Acute water restriction up to 24 hours will result in clinical dehydration, but restriction in excess of 24 hours is not permitted. Once an animal has learned a behavior, the daily amount of fluid provided should be increased to the maximum level that will ensure appropriate performance of task.
- J. Animals should be provided sufficient time to acclimate to food or water regulation/scheduling, at a minimum 3 days, sometimes several weeks, to mitigate stress response unless specifically approved by the IACUC. Consideration should be made to allow food and water to be available concurrently, as rodents typically do not eat without available water. For animals undergoing regulation/scheduling, access to food and/or water must be for 1 hour

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- at a minimum. Regulated levels of food should not be lower than 30% of ad libitum values.
- K. Regulation is not recommended in mice under 8 weeks of age and rats under 12 weeks of age. If still necessary, a strong scientific justification needs to be provided.
- L. Animals on food or fluid regulation/scheduling must be monitored and weighed regularly, at a minimum 3 times a week or more often if stipulated by the IACUC. This needs to be documented.
- M. Specific humane endpoints that would require intervention must be clearly stated in the protocol, including when to return to ad libitum food/water.

V. Definitions

- 1. <u>Ad libitum</u>: Rodents are offered access to a continuous supply of food and water and can eat and drink as much and as often as they want. This is the current husbandry practice at Florida Atlantic University.
- 2. Deprivation: Complete withholding of food or water.
- 3. <u>Regulation/Scheduling</u>: The act of controlling the amount of water and food an animal receives and when the animal receives it. The amount is less than the average daily ad libitum water and food consumption.

VI. <u>Accountability</u>

The Principal Investigator (PI) will be responsible for:

- Assuring that procedures are performed as described in the corresponding IACUC protocol and if necessary submitting an amendment to the protocol and awaiting approval before new method(s) is introduced.
- Assuring that the restricted food and/or fluid is provided at the amount and frequency indicated in the IACUC protocol including on weekends and holidays.
- Monitoring the animals at indicated intervals and assuring recordkeeping requirements.
- Reporting any health concerns resulting from procedure to veterinarian(s)

The IACUC will be responsible for:

- Reviewing and approving, requiring modifications in (to secure approval) or withholding approval of IACUC protocols and/or amendments.
- Providing oversight for all animal procedures conducted including proper procurement and acclimation of animals to observe animal welfare and highquality involving animal research models.
- Monitoring of records for Diet/Fluid regulation/restrictions (during semi-annual inspections or as needed)

The Research Integrity office will be responsible for:

- Administrative support of the IACUC members to facilitate their regulatory function
- Maintaining policy and assure regular review and update as necessary by the IACUC.

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The Attending Veterinarian (AV) or designee will be responsible for:

- Providing Investigators with appropriate consultation in the development of their proposed studies to adhere to this policy.
- Ensure adequate oversight of food/fluid regulated animals including health surveillance, assessment of well-being of animals on a case-by-case basis and evaluation of recordkeeping.
- Reporting any concerns to the IACUC

VII. Procedures

- A. Scientific Justification must be provided in the corresponding IACUC protocol for using regulation/scheduling of food or fluid in rodents. The following needs to be addressed and approved by the IACUC before implementation:
 - Explanation as to why alternatives cannot be used, i.e. reasons why
 positive reinforcement with highly preferred food or fluid cannot be
 considered.
 - 2. Rationale for the level and length of time the regulation/scheduling is proposed.
 - 3. Periods of restriction of food/fluid intake and periods of *ad libitum* feeding/watering.
 - 4. Level of food/fluid regulation, e.g. the formula used for the proposed regulation can be "Food regulation equal to 70% of ad libitum intake until the rodent is at 80% body weight of peer controls."
 - 5. Monitoring frequency and endpoints for intervention.
- B. Animals must be monitored and weighed 3 times per week during times of food/fluid restriction. A baseline weight needs to be taken before implementing the food/fluid restriction (see <u>Fluid and Food Restriction Record Sheet</u> for an example form to use as a monitoring record sheet). In certain studies, additionally a body condition score (BCS) should also be used to evaluate the animals (see <u>Body Condition Scoring (BCS) Guide</u> for a diagram explaining a BCS system).
- C. It is the responsibility of the research staff to provide the food and/or water if restricted. The restriction will be conveyed to the animal care staff through an individual cage label system, i.e. "Special Diet/H₂0" cards (see appendix A). It is the responsibility of the research staff to verify via dating/initialing the card that food/water has been provided. If an animal is found without food or water and no documentation is provided according to this Policy a reasonable attempt will be made to contact the investigator. If the PI or his/her designee cannot be contacted in a reasonable time, then CM personnel will provide the animal with food or water, respectively as applicable.
- D. Records need to be kept in the animal room or close to housing rack easily accessible for CM and research personnel at any time. Records need to reflect the following as applicable:
 - 1. General information (Protocol number, PI< etc.)
 - 2. Date
 - 3. Baseline weight prior to start of diet modification
 - 4. Indication water is given daily if restricted
 - 5. Indication food was given daily if restricted

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- 6. Period of ad libitum food/water access
- 7. Weight and/or BCS
- 8. Health status of the animal
- 9. Any adverse effects observed
- 10. Initials/signature of observer

VIII. <u>Policy Renewal Date</u> 5/26/2026

IX. References

- 1. Bachmanov, AA, Beauchamp, GK and Tordoff, MG (2002). Voluntary consumption of NaCl, KCl, CaCl₂ and NH₄Cl solutions by 28 mouse strains. *Behav. Genet.* 32: 445-457.
- 2. 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
- 3. Tomiyama AJ, Mann T, Vinas D, Hunger JM, Dejager J, Taylor SE. (2010). Low calorie dieting increases cortisol. *Psychosom Med* 72: 357-64.
- 4. Hotz MM, Connolly MS, and Lynch, CB (1987). Adaptation to daily meal-timing and its effect on circadian temperature rhythms in two inbred strains of mice. *Behav Genet* 17(1): 37-51.
- 5. Van Leeuwen SD, Bonne OB, Avraham Y, and Berry EM (1997). Separation as a new animal model for self-induced weight loss. *Physiol Behav* 62(1): 77-81.

POLICY APPROVAL	
Initiating Authority	
Signature:	Date:
Name: Gregg B. Fields, Ph.D., Interim Vice President for Rese	arch

Executed signature pages are available in the Initiating Authority Office(s)

Appendix A

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DIET(v one): Restriction* Special Order(specify):					
H2O(v one): APAP TapH2O Oth Start date:			End date:		
Date	Initials	Date	Initials	Date	Initials
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	Responsible	Party	S	pecial Considera	tions