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Food and Water Restriction in Research Rodents	
Procedures	

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1. Overview

1.1 Purpose

This procedure's purpose is to guide principal investigators and their staff in withholding feed and/or water from laboratory rodents.

The goals of these procedures are to ensure that:

- (a) dietary restrictions are well justified and necessary for study objectives and
- (b) animals subjected to dietary restriction are appropriately monitored.

1.2 Scope

This procedure defines guidelines for research staff who intend to withhold feed and/or water from laboratory animals in their approved IACUC protocol.

Common experimental protocols that require food/water restriction include:

- 1) Behavior study protocols in which withholding food and/or water is required to train animals to perform a task, while providing food or water as a reward for the correct behavior.
- 2) Nutrition studies that require altering levels of specific nutrients in the daily diet.
- 3) Limit-feeding, which is common for some sedentary laboratory animals in order to control obesity or maximize lifespan (such as maintaining 85% of body weight).

2. Definitions

Food or fluid restriction: Any reduction in access to food or water below normal husbandry practices (i.e., *ad lib* access to both). This excludes certain clinical situations under the direction of the veterinarian such as altered diets that do not involve restriction (high/low fat diet, medicated food/water, etc.).

Skin turgor: degree of elasticity in the skin. Skin that has lost elasticity, i.e., doesn't 'snap' back after being pinched, is caused by dehydration.

Body Condition Score: a visual assessment of an animal's weight relative to age, strain and stock based on the amount of tissue cover between the points of the hip, over the transverse processes of the lumbar vertebrae, the cover over the ribs, and the pin bones below the tail. Scoring is done on a scale of 1 to 5, with 1 being emaciated and 5 being obese.



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3. Procedures

3.1 General Guidance

- 1) Food/water restriction (or modification) must be approved by the IACUC and justified based on the scientific objectives of the study. The least amount of restriction that will achieve the scientific objectives must be used.
- 2) Baseline body weight of animal(s) must be recorded <u>before</u> food or water restriction occurs. Body weight loss should be adjusted to normal body weight gain for age matched animals.
- 3) Restriction must be based on a measurable parameter, such as percentage of *ad lib* intake or duration of water restriction.
- 4) In a conditioned-response research protocol, use of a preferred food or fluid as positive reinforcement is recommended.
- 5) Experimental endpoints, clinical symptoms, and conditions for temporary or permanent removal of an animal from a study must be described in the IACUC application. Examples include:
 - a) Body weight loss of greater than 15%
 - b) Appearance (sunken eyes, body condition score of 2 or 1 out of 5)
 - c) Behavior (listless, lethargic)
 - d) Other health issues
 - e) Failure for growing animals to gain weight.
- 6) It is important to note that restriction of water will concurrently cause a rodent to eat less and vice versa.
- 7) Animals co- or group-housed must be observed when feed/water is given to ensure all animals are receiving adequate intake.

3.2 Water Restriction

- 1) When using fluid rewards as motivation for task performance, it is imperative for the investigator to ensure that the daily requirements to maintain a healthy state are met by the sum of earned rewards and supplemental fluid offered.
- 2) Skin turgor must be used to assess hydration status. When the skin is 'tented,' it should snap back quickly. If the tent of skin does not snap back, this indicates at least a 5% dehydration status.
- 3) Animals that have weight loss of 10% from baseline weight are considered clinically dehydrated and should be allowed to freely drink water without interruption. In addition, 0.5-1ml (mouse) or 2-3ml (rat) of subcutaneous warmed isotonic saline (0.9% NaCl) must be administered and the veterinary staff consulted.



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3.3 Food Restriction

- 1) An animal should not lose more than 15% of its baseline weight during a food restriction study. Body weight loss should be adjusted to normal body weight gain for age matched animals.
 - a) Please refer to <u>Charles River Laboratories</u> website to find growth rate charts for the rodents involved in your study. Below are growth rate charts of two commonly used strains at Drexel University.







i) C57BL/6 Mice



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2) Average feed consumption amounts are determined based on age, sex, stock, and strain of animal and should be established before beginning any restriction study.

4. Quality Control & Assurance

4.1 Monitoring and Documentation

- 1) Each cage must be marked with "food/water restriction."
- 2) A <u>daily log sheet must be maintained</u> for each cage of animals on restricted food/water protocols and kept in the animal holding room for the duration of the study. PI's can format their own food/fluid restriction log sheet. An example of a recommended log sheet available for use is included at the end of this policy. Should the sheet fail to indicate that the animal has received its daily ration of food or water, the ULAR staff will attempt to contact the lab and/or provide ad lib food/water if unable to determine if the animal has received its daily ration.
- 3) The log sheet must include:
 - a) Current approved IACUC protocol number
 - b) Investigator name
 - c) Type of restriction
 - d) Animal identification
 - e) Amount of food/water given, including amount of time allowed to eat/drink, if applicable

It is recommended that log sheets include the following information if not maintained in other records. If maintained in other records, these records should be made available to the IACUC upon request:

- a) Animals' initial body weight
- b) Daily/weekly body weights
- c) Daily/weekly body condition score
- d) Presence/Absence of urine and feces (for fluid restriction only)
- e) Skin turgor (for fluid restriction only)
- 4) For animals on lifetime food restriction, such as maintaining 85% body weight based on growth curve for the strain and species, animals must be weighed 2-3 times weekly.
- 5) Water-deprived/restricted animals must be monitored at least once daily, including weekends and holidays, by skin turgor testing.
- 6) Research staff is responsible for monitoring all animals on food/water restriction studies.
- 7) Research staff must be trained and competent to evaluate an animal's condition.
- 8) Veterinary staff and/or IACUC have the right to request the log sheets for review at any time.



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5. Responsibilities

5.1 Drexel University IACUC Responsibilities

The Drexel University IACUC and the IACUC Office are responsible for maintaining this guidance document, training, and monitoring. All exceptions to this policy must be approved by the IACUC. For inquiries regarding these procedures, please contact the Director of Animal Welfare, a part of the Office for Research & Innovation (ORI), or the Attending Veterinarian.

5.2 Principal Investigator Responsibilities

The Investigator is responsible for ensuring that everyone working on an applicable protocol adheres to this policy, monitors all animals on food/water restriction studies, and are trained and competent to evaluate animal's condition.

6. Revisions

Edition 001/Effective Date: 08/2015 – Original Document Edition 001/Review Date: 05/2018 Edition 001/Review Date: 02/2021 Edition 002/Revision Date: 03/13/2024 and Effective Date 04/09/2024 – Revised Document

- Updated formatting to new template.
- Modified title to "Food and Water Restriction in Research Rodents Procedures"
- Modified "food or fluid restriction" definition.
- Added growth chart graphs and resource link to Charles River Laboratories.
- Addition of Drexel IACUC Responsibilities.
- Modified log form example template columns.

Drexel University Food/Fluid Regulation Monitoring Form for Rodents

IACUC Protocol Number:	Cage ID (if applicable):
Principal Investigator:	Procedure Date:
Responsible Research Staff Name:	Species: Mice Rats
Lab Phone:	Procedure: Fluid Restriction
Drexel Email:	Food Restriction
After Hours Contact:	

Animal ID:

Date	Time	Animal ID	Body Condition: BCS	Body Condition: B.Wt. (g)	Hydration: Urine/Feces	Hydration: Skin Turgor	Food Regulation: Amount of Food Given & Duration Offered	Fluid Regulation: Fluids Given (ml) & Duration Offered	Initial

Body Condition Score (BCS): 1(emaciated)-5(obese) Skin Turgor: N=Normal, A=Abnormal

This form is an example, and PI's are able to use either this form or their own, to monitor their animals on Food/Fluid regulation, as long as the documentation of monitoring is available in the animal housing room for ULAR staff. This form can be used to monitor more than one animal. After all the rodents listed on this sheet are euthanized or have died, this sheet should be kept on file in the lab. Frequency of monitoring must be done according to the approved IACUC protocol dealing with the experimental condition under study.