
1. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide information and guidelines in regard to food and water restriction in rodent experiments.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinarian, veterinary care staff.

3. INTRODUCTION

- 3.1. Food and water restriction includes any deviation from usual rodent husbandry procedures (*ad lib* food and water). The restriction process may include the strict food and/or water monitoring and control, as well as the scheduled access to food or fluid sources, both of which are performed by the research staff.
- 3.2. Rodents have a very high metabolism and gastrointestinal transit time. Therefore, they can be affected very quickly and significantly from food and water restriction.
- 3.3. Special attention should be given to ensure that animals consume a balanced diet. Food consumption may also decrease with water restriction.
- 3.4. Experimental reasons for introducing food and/or water restriction include, but are not limited to, studies that use food and/or water as a motivational tool in training rodents to perform novel or learned tasks required for a research protocol.
 - 3.4.1. In the case of conditioned-response experiments, use of a highly preferred food or fluid as a positive reinforcement instead of restriction is recommended. Where possible, a reward strategy, e.g., highly preferred food, should be used to motivate an animal rather than using deprivation.
 - 3.4.2. Deprivation or restriction of resources should only be used when there is no alternative.
- 3.5. Prior to proceeding with food and/or water restriction, consider:
 - 3.5.1. Finding alternatives to food restriction, e.g., food reward, whenever possible.
 - 3.5.2. Finding alternatives to water restriction, e.g., citric acid water, whenever possible.
 - 3.5.3. If the PI can demonstrate that there are no suitable alternatives to food restriction, the minimal restriction to achieve experimental results needs to be used. Where possible, pilot studies should also be carried out to determine whether food or water regulation is essential.
 - 3.5.4. Food restriction needs to be selected over water restriction whenever possible.
 - 3.5.5. Potentially adverse outcomes and criteria for removal of the animal from the experiment (humane intervention points) must be addressed.
 - 3.5.6. Methods used to assess animal health and well-being must be determined, e.g., body weight, body condition score, hydration status.

4. PROCEDURES

- 4.1. Scientific justification for food and/or water restriction must be provided in the Animal Use Protocol (AUP) and approved by the Facility Animal Care Committee (FACC). Purpose and planned duration of food or water restriction must be specified. The justification must address the principles listed in section 3.6.
- 4.2. Research staff must be trained and competent to evaluate the animal's condition.
- 4.3. The goal is to establish the least food and/or water restriction necessary that will produce the required behavioral performance or experimental results for the study while maintaining animal well-being.
- 4.4. Establishment of food and water consumption baselines:

- 4.4.1. Prior to food or water restriction, the animal's baseline body weight must be recorded. All other weights will be compared to this baseline body weight.
- 4.4.2. Estimated daily fluid maintenance requirement in rodents:
 - 4.4.2.1. Mice: 3.9 to 8.2 ml/mouse/day
 - 4.4.2.2. Rats: 80 to 125 ml/ kg body weight
 - 4.4.2.3. Species and strain variation are common, and the body weight, body score condition and hydration status must be evaluated daily for each animal on water restriction.
- 4.4.3. Estimated food consumption in rodents:
 - 4.4.3.1. Mice: 120-180 g/kg/day of a nutritionally balanced diet containing 16-20% protein and 5-25% fat, which can be calculated to 3-4.5 g of food for a 25g mouse.
 - 4.4.3.2. Rats: 50-60 g/kg/day of a nutritionally balanced diet containing 12-27% protein and 5-25% fat, which can be calculated to 15-18g of food for a 300g rat.
 - 4.4.3.3. Species and strain variation are common, and the body weight and body condition score must be evaluated frequently for each animal on food restriction.
- 4.5. Food/Water Restriction in young or growing animals:
 - 4.5.1. Young or growing animals are especially sensitive to fluid restriction and malnutrition. Special concern for their health and minimum growth requirements must be met.
 - 4.5.2. Body weight should not be compared with the animal's baseline body weight. Instead, animals should be maintained within a specific percentage (i.e., 85%) of an age/sex/strain-matched control littermate with *ad libitum* food and water. If no control littermates are present, vendor growth charts can also be used as comparison.
- 4.6. Implementation of food or water restriction:
 - 4.6.1. Animals undergoing surgeries must be fed *ad libitum* for 1 week before the start of the food or water restriction, i.e., for 1 week after the surgical procedure.
 - 4.6.2. When fasting animals, the shortest possible period should be used, preferably during the light phase, as rodents typically consume a large part of their daily food intake at night.
 - 4.6.3. Animals must be acclimated gradually, over a period of 3 to 7 days, to new restriction paradigm.
 - 4.6.4. Body condition and hydration status must be assessed frequently by the research staff.
 - 4.6.4.1. Animals under food restriction must be weighed at least twice a week.
 - 4.6.4.2. Animals under water restriction must be weighed daily.
 - 4.6.5. No rodent can be completely deprived of water/fluids for more than 24 hours.
 - 4.6.5.3. For scheduled access of water, the animals are given *ad libitum* access to water for a minimum of 1 hour, at least once every 12 hours for mice and once every 24 hours for rats.
 - 4.6.5.4. When water restriction is based on volume per day, mice that do not drink the minimum established water volume during training must be given the remaining water after training.
 - 4.6.6. Food should be provided concurrently with water as the animal benefits from simultaneous availability of food and water.
 - 4.6.7. Animals may be separated during feeding to avoid competitive behavior in socially-housed conditions. If an additional cage is needed for separation, the temporary cage must be labeled with an appropriate temporary cage card with the identification of the animal, the date and time of the separation, and contact information.
 - 4.6.8. Investigators are encouraged to offer alternative rewards (e.g., fruit juices, raisins, peanuts, etc.) allowing the animal to increase the restriction baseline while still maintaining task performance.
- 4.7. Withdrawal of food or water control:
 - 4.7.1. Withdrawal of food or water restriction should occur when testing is no longer required for extended periods of time.
 - 4.7.2. Once the long-term restriction protocol is no longer required, animals must be brought back to *ad libitum* food and water, gradually over a period of 3 days. During this time, the animals should be monitored closely for deleterious effects of fluid overload and gastro-intestinal problems.

- 4.8. Ongoing health monitoring:
 - 4.8.1. Chronic food or water restriction results in the establishment of new physiological set points.
 - 4.8.2. The following parameters must be monitored as specified below:
 - 4.8.2.1. Body weight: Animals should be weighed at least twice weekly, in no less than 48-hour intervals. The body weights must be obtained in as consistent a manner as possible, e.g., at the same time each day.
 - 4.8.2.2. Body condition and hydration status: research staff should perform assessment through qualitative observation of body condition score, presence of fresh feces, and general mental status. Please refer to Body Condition Score Outline for rats and mice.
 - 4.8.2.3. Animals exhibiting clinical signs are to be reported to veterinary care staff,
- 4.9. Record keeping:
 - 4.9.1. Each cage must be identified with “Food or Water Restriction” label, i.e., Fed by Investigator, Water given by Investigator, Water Deprivation, Food Deprivation.
 - 4.9.2. Rodents on food and/or water restriction must be monitored daily, and food or water intake must be recorded daily in the Food or Water Restriction Log and kept in the animal holding room. A log sheet is required for all restrictions lasting more than 12 hours. The log on page 4 illustrates the information required on each log sheet.
 - 4.9.3. If the log sheet is not signed for the previous day, the emergency number of pellets is given to the animal on food restriction and the emergency volume of water is given to the animal on water restriction.
 - 4.9.4. Individual records must be maintained and include the following:
 - 4.9.4.1. Body Weights
 - 4.9.4.2. Hydration status
 - 4.9.4.3. Food or water consumption data: Number of pellets or ml of water consumed daily, or amount of time permitted with free access to food or water.
- 4.10. Termination of food or water restriction:
 - 4.10.1. The following criteria will necessitate evaluation by the veterinarian:
 - 4.10.1.1. Significant weight loss, e.g., exceeding 15% of baseline body weight, at any time throughout the study.
 - 4.10.1.2. Body condition score of 2 or less.
 - 4.10.1.3. Severe dehydration
 - 4.10.1.4. Abnormal behavior (increased aggression, ruffled fur, recurrent episodes of self-harming, depression) that has not improved despite veterinary intervention.
 - 4.10.2. Upon consultation with the veterinarian, food or water restriction may be reduced or stopped.
 - 4.10.3. Food and/or water restriction can only resume with approval from the veterinarian.

5. REFERENCES

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- 5.2. Carpenter JW. Exotic Animal Formulary 4th edition. Elsevier, St-Louis, Missouri. 2013.
- 5.3. Rowland, NE. Food and Fluid restriction in common laboratory animals: balancing welfare considerations with Scientific Injury. *Comparative Medicine*, 57 (2): April 2007.
- 5.4. Toth L.A., Gardiner T.W. “Food and water restriction protocols: physiological and behavioral considerations”. *Contemp. Top. Lab. Animal Sci.* 39(6), 2000.
- 5.5. Hickman, DL and Swan, M. “Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease”. *Journal of the American Association for Laboratory Animal Science*, 49(2), pages 155-159: March 2010.

- 5.6. Ullman-Culleré, M. H., and Foltz, C. J. "Body condition scoring: a rapid and accurate method for assessing health status in mice". *Comparative Medicine*, 49(3), pages 319-323: 1999.
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SOP REVISION HISTORY

DATE	NEW VERSION
2017.01.25	4.6.1. Animals undergoing surgeries must be fed ad libitum for 1 week before the start of the food or water restriction, i.e., for 1 week after the surgical procedure.
2017.01.25	4.7.1. Withdrawal of food or water restriction should generally occur when testing is no longer required for extended periods of time.
2019.03.27	4.4.3. No rodent can be completely deprived of water/fluids for more than 24 hours. For scheduled access of water, the animals are given ad libitum access to water for a minimum of 1 hour, at least once every 12 hours for mice and once every 24 hours for rats.
2019.03.27	4.4.4. The estimated food consumption for a mouse is 120-180g/kg/day of a nutritionally balanced diet containing 16-20% protein and 5-25% fat, which can be calculated to 3-4.5g of food for a 25g mouse. The estimated food consumption for a rat is 50-60g/kg/day of a nutritionally balanced diet containing 12-27% protein and 5-25% fat, which can be calculated to 15-18g of food for a 300g rat. However, species and strain variation are common and the body condition score must be evaluated daily frequently for each animal on food restriction.
2019.03.27	4.6.3. Body condition and hydration status must be assessed daily frequently by the research staff. Animals must be weighed at least twice a week.
2019.03.27	4.8.2.2. Body condition and hydration status: Once daily , research staff must perform assessment through qualitative observation of body condition score, presence of fresh feces, and general mental status. Please refer to Body Condition Score Outline for rats and mice.
2019.03.31	Log updated
2023.04.03	2. RESPONSIBILITY Principal investigator (PI) and their research staff, veterinarian , veterinary care staff.
2023.04.03	3.5. In the case of conditioned-response experiments, use of a highly preferred food or fluid as a positive reinforcement instead of restriction is recommended. Deprivation or restriction of resources should only be used when there is no alternative. Where possible, a reward strategy, e.g., highly preferred food, should be used to motivate an animal rather than using deprivation.
2023.04.03	3.6. Prior to proceeding with food and/or water restriction, consider: 3.6.1. Finding alternatives to food restriction, e.g., food reward, whenever possible. 3.6.2. Finding alternatives to water restriction, e.g., citric acid water, whenever possible. 3.6.3. If the PI can demonstrate that there are no suitable alternatives to food restriction, the minimal restriction to achieve experimental results needs to be used. Where possible, pilot studies should also be carried out to determine whether food or water regulation is essential. 3.6.4. Food restriction needs to be selected over water restriction whenever possible. 3.6.5. Potentially adverse outcomes and criteria for removal of the animal from the experiment (humane intervention points) must be addressed. 3.6.6. Methods used to assess animal health and well-being must be determined, e.g., body weight, body condition score, hydration status.
2023.04.03	4.4.1. The estimated daily fluid maintenance requirement in rodents (mouse and rat) is 100 ml/kg/day. However. 4.4.1. Estimated daily fluid maintenance requirement in rodents: 4.4.1.1. Mice: 3.9 to 8.2 ml/mouse/day 4.4.1.2. Rats: 80 to 125 ml/ kg body weight 4.4.1.3. Species and strain variation are common, and the body weight, body score condition and hydration status must be evaluated daily for each animal on water restriction. 4.4.2. Estimated food consumption in rodents: 4.4.2.4. Mice: 120-180 g/kg/day of a nutritionally balanced diet containing 16-20% protein and 5-25% fat, which can be calculated to 3-4.5 g of food for a 25g mouse. 4.4.2.5. Rats: 50-60 g/kg/day of a nutritionally balanced diet containing 12-27% protein and 5-25% fat, which can be calculated to 15-18g of food for a 300g rat. 4.4.2.6. Species and strain variation are common, and the body weight and body condition score must be evaluated frequently for each animal on food restriction.
2023.04.03	4.5.1. Young or growing animals (<14 weeks) are especially sensitive to fluid restriction and malnutrition. Special concern for their health and minimum growth requirements must be met.
2023.04.03	4.6.2. When fasting animals, the shortest possible period should be used, preferably during the light phase, as rodents typically consume a large part of their daily food intake at night.
2023.04.03	4.6.4. Body condition and hydration status must be assessed frequently by the research staff. 4.6.4.7. Animals under food restriction must be weighed at least twice a week. 4.6.4.8. Animals under water restriction must be weighed daily.
2023.04.03	4.9.4.2. Hydration status
2023.04.03	4.10.1.5. Body condition score < of 2 or less.
2023.04.03	4.10.1.6. Severe dehydration

