Guidelines on Experimental Food or Water Restriction or Manipulation in Laboratory Animals

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1. Purpose

Food and water restriction can be an important and commonly used research tool in many experimental studies. This restriction has the potential to adversely affect an animal’s well-being. In keeping within federal and university guidelines, and policies to minimize pain, distress and discomfort of animals used in research, the following guidelines have been developed for implementing food or water restriction or manipulation in experimental studies involving animals.

Pre-anesthetic fasting (with access to water) does not fall under these guidelines. See the species-specific anesthesia and anesthesia guidelines for recommendations on this topic.

In a natural environment, it is not uncommon for animals to have restricted access to food and/or water throughout the day. “Working” for a food reward is also not a completely foreign activity, as animals in nature must forage or hunt for their food. As long as the periods of deprivation do not exceed the ability of homeostatic mechanisms to compensate, these animals are able to carry out normal activities. Common purposes for using food or water deprivation or restriction:

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1. Studies pertaining to metabolism, energy regulation, fluid balance, etc.
2. Studies pertaining to mechanisms and motivations of hunger and/or thirst.
3. Studies using hunger or thirst as a motivating force to perform behavioral tasks.
4. To promote a healthier body condition in an obese or sedentary animal.
5. Studies analyzing the effects of restriction or manipulation on aging or cancer.
6. Prior to surgery, to prevent complications such as vomiting and aspiration.

2. Definitions

1. **Restriction**: A limitation placed on an animal’s access to food or water. It can be described in terms of either the amount of food or water provided on a daily basis (volume or weight) or the amount of time daily that an animal is given access to food and/or water.
2. **Manipulation**: A change in the composition of the normally offered food or water. This can include addition or deletion of a nutrient in an animal diet and/or addition of a compound, medication or other substance to the food or drinking water provided to the animal.

3. Procedures

1. When manipulation of a diet occurs:
   1. Deviations from the standard diet must be described.
   2. The nutritional composition and food palatability should be conducive to animal wellbeing, unless scientifically justified in the protocol.
2. When using food or fluid restriction to provide motivation, consider whether the use of highly preferred food or fluid as positive reinforcement can be used instead of restriction.
   1. If restriction is necessary, use “the least restriction that will achieve the scientific objective while maintaining animal well-being,” understanding that multiple factors including “species, strain, or age and sex of the animals” can “influence the amount of food or fluid restriction that can be safely used in a given protocol”. (From The Guide).
3. In order to determine the appropriate level of food/fluid restriction, it is necessary to know the normal quantities of food that are required for the maintenance of the species in question. Life stage (growth, pregnancy, lactation, geriatric, etc.) and state of health must also be taken in consideration when determining maintenance requirements.
4. Non-ruminant animals that do not have access to food or water for longer than the periods outlined below are defined as restricted.

1. **Table 1: Food**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Species</th>
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<tr>
<td>Twice daily</td>
<td>Non-human primates (&gt;1 yr old)</td>
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5. Food and water provisions to animals outside of the above tables (e.g. a fast of 6 hours or longer in rodents) constitute restriction and must adhere to the following:

1. Description within the animal use protocol:
   1. Describe the method and duration of restriction (complete restriction for a set period, vs. a percentage of the animal’s daily ration over a period of time).
   2. Scientifically justify the food and/or fluid restriction.
   3. List any research specific end points related to the restriction and the course of action (euthanasia, intervention, removal from study) for animals that reach those endpoints.
   4. If animals are receiving their daily ration of food/water as part of the experimental procedure, the approved protocol must clearly define when and how food and water will be provided on days when no experiments are conducted.
   5. Describe the method and frequency of monitoring in order to assure animal health.
      1. Diligent record keeping on daily food or fluid consumed, hydration status, appearance, general affect, experimental performance, and routine body weights as applicable should be readily available for review by the veterinary staff and IACUC. (See section 3.g)

2. Deviations from standard chow must be described in the animal use protocol. The investigator is responsible for assuring that specially formulated diets are nutritionally adequate and palatable unless the scientific needs of the research protocol necessitate otherwise. If this is the case, the investigator must describe the adverse effects of the diet.

3. Acclimate animals to new restriction paradigms gradually. Ideally, during acclimation, the animal should not lose more than 10% body weight in a week.

4. Special considerations for water restriction
   1. To ensure that the total amount of water an animal receives is adequate to meet daily needs, it is common to either allow the animal to work to satiation during the experiment or to provide a period of free access to fluid after the experiment.
   2. Animals often drink to satiation within the first 30 minutes of access to a water source even if it is available for longer.
   3. When water is restricted, food consumption will also often decrease. Therefore food should be available during periods when water is provided or shortly after, to encourage eating. This will also allow animals to consume solutes to help maintain fluids in circulation.

6. Upon review of the animal use protocol, non-ruminant animals deprived of food for longer than 24 hours (48 hours for ruminants), or of water for 24 hours for any species may be considered prolonged restriction and placed in Category E.

7. Monitoring: methods and frequency
   1. % Body Weight (BW) either as a % from when animal was fed ad libitum prior to study or as compared to a matched conspecific.
   1. For food regulation studies, an animal’s BW should not decrease by more than 20% of their pre-restricted body weight, as indicated by the End-Stage Illness Scoring System. If it does, the animal should be euthanized or placed back onto the normal diet for that species. The weight should be recorded as outlined in the IACUC Animal Use Protocol. The ULAM veterinary staff recommends weighing animals at least daily.
      1. Body weights should be recorded at least weekly and more often for animals requiring greater restrictions (The Guide, 8th ed.).
      2. Measure body weights at physiologically appropriate times (i.e. before and after periods of water deprivation may be useful)
      3. The scales used to weigh animals must be properly calibrated and this should be checked periodically throughout the study (e.g. monthly)
   2. Calculation of 20% loss of BW
      1. Obese animals: Should be calculated from ideal BW, not starting obese weight.
1. If an animal is obese and on calorie restriction, but is intended to be used in a restriction protocol, the weight should be initially recorded when the animal reaches an “optimum” healthy weight. Subsequent weight loss calculations should be derived from the initial optimum healthy weight not the starting obese weight.

2. **Growing Animals:** Should be based on normal weights found in established growth rate curves or the growth rate of a matched unrestricted control animal.
   1. If the animal in question is growing, its growth rate as compared to a matched unrestricted control or a known growth curve can be used for monitoring. An animal should not be more than 20% below normal weight for that age group.

2. Measuring amount of food/fluid consumed
   1. The amount earned during experimentation plus any other “free” food or water provided must total to an amount sufficient to maintain the animal in a healthy state.
   2. Fluids must at least meet daily maintenance amounts, which vary widely by species, strain, environment, and efficiency of fluid-saving mechanisms. The animal must be provided with enough fluid to replace calculated daily losses, which may vary depending on the physiology of the animal (diabetes, etc).
   3. Daily recording of food or fluid intake amounts is recommended.
   4. The diet should still be complete and balanced to meet the species’ need (e.g. still providing a source of Vit C to NHPs).

3. Solid and fluid waste output and moistness of feces
   1. Animals adapting to food or fluid restriction may produce concentrated urine or have diminished quantities of excrement that are less moist than normal. Absence of urine or feces (e.g. few or no fecal pellets for > 24 hours) indicates inadequate intake and should be reported to the veterinary staff.

4. Clinical signs of distress
   1. An animal with a Body Condition Score (BCS) of 2/5 or less is considered under conditioned, and should be reported to ULAM veterinary staff. This scoring system can be found in the End-Stage Illness Scoring System.
   2. The texture and elasticity of the skin are important indicators of an animal's hydration status.
      1. The skin should return to normal position rapidly after being pinched. If the skin does not return to its normal position the animal is considered dehydrated and should be reported to veterinary staff.
   3. Other clinical signs of illness, including lethargy, a rough coat, sunken eyes, dry mucous membranes, and poor performance on behavioral tests should be reported to the veterinary staff.

5. Chemistry values
   1. Serum protein levels, albumin, osmolality, BUN/Creatinine, electrolyte values, ketone levels or other acid-base imbalances indicating metabolic acidosis may be helpful in monitoring the health status of a fasted or water deprived animal. Urine specific gravity may also help establish the kidney health of water-deprived animals.

8. Other considerations
   1. For animals with specific conditions that alter food and water homeostasis (e.g. diabetes, genetic obesity), this condition should be taken into account when reviewing a restriction protocol and the above recommendations may not apply.
   2. Animals on a restricted diet should be allowed a short-term period of unrestricted feeding/drinking prior to a surgical fast to help prevent hypoglycemia or dehydration.
   3. Between experiments, it may be advisable to allow the animal an unrestricted period of feeding/drinking to reestablish baseline weights and hydration status. The intake should still be gradually introduced and carefully monitored to prevent adverse effects from rapid consumption of large amounts of food (e.g. bloat).

4. Related Documents
   1. End-Stage Illness Scoring System
   2. Food and Water References

5. Appendices
   1. Appendix A: **Species Specific Considerations for Food or Water Restriction Deprivation** (When Used for Behavioral Modification Studies)