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DEFINITION OF TERMS

Prior discussions of alteration in food and water access for laboratory animals focused on *restriction*. Recent adoption of the 8th Edition of the *Guide for the Care and Use of Laboratory Animals* (NRC 2011) has suggested that a more appropriate term is *regulation*. Regulation of food or fluid intake may be required for the conduct of some physiological, neuroscience, and behavioral research protocols. The regulation process may entail *scheduled access* to food or fluid sources, so an animal consumes as much as desired at regular intervals, or *restriction*, in which the total volume of food or fluid consumed, is strictly monitored and controlled (NRC 2003). The objective when these studies are being planned and executed should be to use the least restriction necessary to achieve the scientific objective while maintaining animal wellbeing.

PROTOCOL DEVELOPMENT

The development of animal protocols that involve the use of food or water regulation (FWR) requires the evaluation of three factors:

- 1. The necessary level of regulation. Describe how, why and to what extent food or water intake will be limited for animals on the study. The inclusion of a complete and accurate description of all phases of the study will assist the IACUC in its review and help to ensure that the institution remains in compliance with the Animal Welfare Act Regulations (AWARs) during its review (See Table 1)
- 2. Potential adverse consequences of regulation. Describe, in detail, each phase of the study procedure, including the monitoring and training of study animals.
- 3. If animals will be screened for selection into a FWR study, the proposal should describe the screening process.
- 4. Methods for assessing the health and well-being of the animals (NRC 2003).
- 5. In addition, the following factors influence the amount of food or fluid restriction that can be safely used in a specific protocol:
 - a. the species, strain, or stock
 - b. gender, and age of the animals
 - c. thermoregulatory demand
 - d. type of housing
 - e. time of feeding

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- f. nutritive value and fiber content of the diet
- g. prior experimental manipulation

The degree of food or fluid restriction necessary for consistent behavioral performance is influenced by the difficulty of the task, the individual animal (see Figure 2), the motivation required of the animal, and the effectiveness of animal training for a specific protocol-related task.

The animals should be closely monitored to ensure that food and fluid intake meets their nutritional needs. Body weights should be recorded at least weekly and more often for animals requiring greater restrictions (NRC 2003). Written records should be maintained for each animal to document daily food and fluid consumption, hydration status, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from a protocol (NRC 2003). In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended.

Deprivation is defined as follows:

- 1. Water Deprivation (Defined as no intake for more than):
 - a. 8 hours: Lagomorphs
 - b. 23 hours: Rodents
 - c. 28 hours: Ruminants

Note: Provision of 1 hour ad lib water every 12 hours is neither deprivation nor restriction. This would be considered *regulation*

- 2. *Food Deprivation* (Defined as no intake for more than):
 - a. 36 hours: Simple stomach animals
 - b. 72 hours: Ruminants

ENDPOINTS

Specific interventional endpoints must be clearly stated in the protocol.

1. Food Restriction/Scheduling:

For food regulation, a rodent may not lose more than 20% of age-strain-sex matched controls or baseline body weight (if adult). After 20% weight loss has been achieved (the animal weight is 80% of baseline weight or matched controls), the daily food allowance must be increased to prevent additional weight loss. Regulation cannot be attempted again until the animal weighs at least 80% of its original weight.

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A rodent given a body condition score of two or lower must have its daily food allowance increased. Increase the food ration until the animal receives a BCS of two or higher (Figure 1)

2. Fluid Regulation/Scheduling:

Rodents on fluid regulation/scheduling with a weight loss of 10% of baseline weight should be considered clinically dehydrated and should be treated as outline below.

CLINICAL MONITORING AND ANIMAL WELFARE CONSIDERATIONS

Clinical signs associated with possible distress due to FWR include the following:

- 1. Decreased skin turgor
- 2. Dry mucous membranes
- 3. Increased plasma osmolality (Posm = 2[Na+] + [Glucose mg/dl]/18 + [BUN mg/dl]/2.8) Normal=280-303 mosmoles/kg
- 4. Behavior suggestive of extreme thirst or hunger
- 5. Distressed animals might also show behavioral changes such as lethargy, agitation or altered patterns of aggression.

If these signs are more than slight or momentary, personnel can reasonably assume that the animal is in distress and should take appropriate action. Because the hunger drive may be dulled over long periods of time, personnel may observe differences in acute and long-term responses in an animal whose food intake has been restricted.

When planning such a study, a PI should consult with the institution's attending veterinarian and must identify methods that can be used to minimize any discomfort, distress or pain experienced by the animals (AWA section 2.31(d)(1)(iv)(B)).

The AWARs require monitoring procedures to be in place for animals undergoing FWR. At minimum, personnel should monitor body weight, body condition, hydration status and behavioral changes of the animal. Personnel should also record the amount of food or water offered to and consumed by animals. Monitoring of plasma osmolality and electrolyte levels may also be valuable. Before beginning FWR, a PI should record baseline data, including initial body weight and normal behavior patterns, of each animal that will be included in the study.

IACUC OVERSIGHT AND REVIEW CONSIDERATIONS

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The IACUC should consider the following:

- 1. Determine whether the degree of FWR proposed is appropriate for the type of study and is the minimum necessary to achieve the study's desired results.
- 2. Many studies require only slight FWR. Any food or fluid rewards that will be used as positive reinforcement during training or study sessions should be taken into consideration when evaluating the extent of FWR in a study.
- 3. Both the AWARs (section 2.38(f)(2)(ii)) and the *Guide* allow a research facility's IACUC to approve exceptions to the standard feeding and watering requirements for animals in a study, as long as such exceptions are scientifically justified and required for the purposes of the study. Before a Principal Investigator (PI) can proceed with a study employing FWR, the IACUC must approve the procedure as an exception to the standards.
- 4. If the IACUC decides that FWR will likely cause pain or distress to the study animals, the PI must search for and consider using any alternatives. The PI must then list all the methods and sources used to look for alternatives, and the IACUC must be satisfied that the search was adequate.
- 5. While planning the study, the PI and the attending veterinarian should consider who would provide supportive care and what kind of care might be necessary.
- 6. The research facility must then report this approved exception in that year's Annual Report to the USDA. The facility should report whether or not the animals were found to have suffered from distress as a result of the FWR, as required by section 2.36(b) of the AWARs. Animals suffering no distress from the FWR can be reported in Column C. If animals suffer distress that is relieved, the facility should report the animals in Column D. If animals suffer distress that could not be relieved, the animals should be reported in Column E.

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TABLE 1 | MINIMUM FREQUENCIES GIVEN IN THE AWARS $^{\rm 2}$ FOR FEEDING AND WATERING OF ANIMALS REGULATED UNDER THE AWA

Species	Minimum feeding frequency		Minimum watering frequency
Dogs and cats	At least once each day 3.9(a)	Section	Continually available or no less than twice daily for at least 1 h each time Section 3.10
Hamsters and guinea pigs	Each day 3.29(a)	Section	Daily Section 3.30
Rabbits	At least once each day 3.54(a)	Section	Daily Section 3.55
Nonhuman primates	At least once each day 3.82(b)	Section	Continually available or not less than twice daily for at least 1 h each time Section 3.83
Marine mammals	At least once each day 3.105(a)	Section	N/A (required to have pools in which water quality is maintained)
All other regulated animals	At least once each day 3.129(a)	Section	As often as necessary, taking age, species, condition, size and type of animal into consideration Section 3.130
Mice and Rats ^a	Each day		Daily

^aUniversity IACUC policy follows requirements for regulated rodent species

^b Certain hibernating / estivating species do not consume water / feed for periods which would qualify as deprivation, but is in fact 'normal' for the species while hibernating / estivating. The IACUC defines hibernating / estivating animals as 'NORMAL.' Thus, these instances are neither deprived nor restricted. Hibernating / estivating protocols will be reviewed by the IACUC on a case-by-case basis

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Figure 1 | BODY CONDITION SCORE OF RODENTS

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
 - 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



- 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.

FROM: Hickman, D.L., M. Swan 2010. Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease. J Am Assoc Lab Anim Sci 49(2):155-9.

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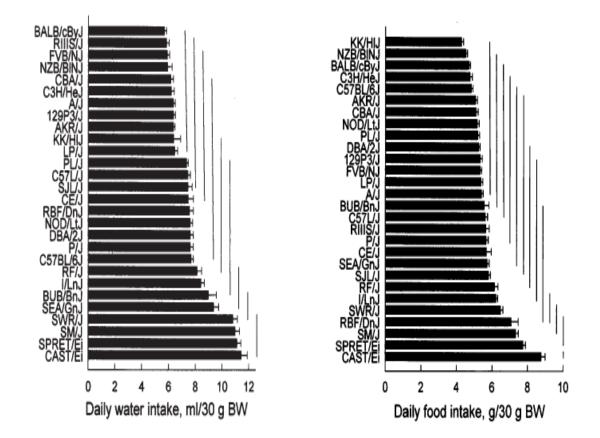


Figure 2| PUBLISHED GRAPHS OF WATER AND FOOD INTAKE OF 28 COMMON MICE STRAINS

Bachmanov, A. A., Beauchamp, G. K., and Tordoff, M. G. (2002). Voluntary consumption of NaCl, KCl, CaCl2 and NH4Cl solutions by 28 mouse strains. *Behav. Genet.* **32**:445–457

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- **2.** National Research Council (2011) Guide for the Care and use of Laboratory Animals, 8th Edition. National Academies Press.
- **3.** National Research Council (2003) Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Academies Press, Washington, DC.)
- **4.** Animal Welfare Act Regulations (2002) United States Department of Agriculture (APHIS)
- Silverman, Jerald, et.al. (2007) The IACUC Handbook, 2nd Edition. CRC Press, Boca Raton, FL