

Guidelines for Diet Control in Laboratory Animals

Introduction:

Diet control includes scheduled access to food or fluid sources in which the animal is given *ad libitum* food and fluid access at regular intervals, or restriction, in which the total volume of food or fluid consumed is strictly monitored and controlled. In both instances, animals should be closely monitored to ensure that their nutritional needs are met. Professional judgment must be used in all situations to ensure the well-being of the animal throughout the period of study.

When normal, healthy animals are provided with *ad libitum* food and fluid sources for at least 48 hours prior to a short duration fast of up to 24 hours, followed by a return to *ad libitum* feeding and watering, this is generally not considered to be diet control. Thus, Institutional Animal Care and Use Committees (ACUCs) do not generally require the frequency and number of such fasts (e.g., prior to surgery or blood or other tissue collection) to be delineated within the Animal Study Proposal (ASP).

All study designs that include diet control must be approved by the individual Institute/Center (IC) ACUC. Each ACUC must evaluate the diet control parameters, monitoring plan, intervention criteria, and pain-distress categorization the animals will experience.

The *Guide* states, “use the least restriction necessary to achieve the scientific objective while maintaining animal well-being.” Some diet control is intrinsic to wild animals, as they must forage, travel extended distances, solve problems, or otherwise work to obtain food and water. Further, the professional judgment of many investigators, veterinarians, and animal behaviorists, is that performing a task for reinforcement is behaviorally enriching for laboratory animals. Therefore, diet control in and of itself is not an unusually harsh imposition upon laboratory animals.

In the development of ASPs utilizing diet control, Principal Investigators (PIs) must address three fundamental issues: 1) the necessary level of regulation/restriction; 2) potential adverse consequences; and 3) methods for assessing health and well-being. Consideration must be given to the species, strain or stock being used and the animal’s sex, size, age, health status, prior experimental manipulations, body condition and/or hydration status, and concurrent treatments. Finally, other factors influencing food and fluid restriction must be considered, such as thermoregulatory demand, type of housing, opportunity to exercise, time of feeding, nutritive value, and content of the diet. Careful attention to these factors can facilitate the establishment of interventional endpoints to maintain the health and well-being of the animals under study. How these factors are considered, and interventional endpoints established will vary with the study objectives. For example, behavioral research often requires that a young and healthy animal perform a task for which it receives food or fluid reinforcement, whereas chronic calorie restriction is an often-used research paradigm in aging studies. These two examples could very well require different interventional endpoints.

Animal health observations must be conducted daily by the animal care or investigative staff. Diligent record keeping of daily food and/or fluid access/intake, volume consumed, hydration status, appearance, general affect, experimental performance, and body weight are reliable means for ensuring that subjects remain in a healthy state. Records should be maintained as appropriate for the paradigm, reviewed regularly, and be accessible for review by the veterinary staff and the ACUC when necessary. In addition, a plan of action, complete with endpoints for therapeutic intervention, should be considered when the experimental protocol is developed.

It is recommended that experimental animals undergoing diet control be weighed at regular intervals. If subjects are used in behavioral studies that employ periodic experimental sessions, it is ideal to weigh subjects before and after each session. Provision of vitamins, minerals, and other supplements may be beneficial in maintaining an animal's clinical condition throughout a diet control paradigm. Written records should be maintained for each animal as stipulated in the *Guide* and in the ASP to document food and fluid consumption, hydration status and any behavioral and clinical changes to be used as criteria for the temporary or permanent removal of an animal from study.

Young, developing animals have additional dietary requirements for maintaining their normal rate of growth. Investigators working with young animals should specifically address in their ASPs their expectations for any retardation of growth rate and/or adult size. Comparisons with litter mates, with similar control animals, or with growth standards will prove useful when assessing growth. In many situations, when caloric or fluid restriction has been justified in developing animals, animals will not reach their projected adult size. However, in all other respects, they will develop into normal adults. Body condition scoring is a useful way to assess young/growing animals, as these animals may not lose body weight when the diet is restricted but may lose condition (i.e., muscle and fat reserves) while increasing in height/body length. Measuring weight alone will miss such changes. In all situations, young, developing animals on restricted food or fluid regimens should be carefully monitored as stipulated in the ASP.

Food Regulation:

CALORIE RESTRICTION: Although *ad libitum* feeding is the normal practice in the vivarium, it is not the norm for most animals in the wild. Many animals used in biomedical research are sedentary, obese, glucose intolerant, and have other morbidities. The *Guide* states, "Animals should be fed palatable, uncontaminated diets that meet their nutritional and behavioral needs at least daily, or according to their particular requirements, unless the protocol in which they are being used requires otherwise." However, every-other-day feeding (unlimited access to regular food pellets, on an alternate day feeding schedule) and paired feeding (equal proportions of reduced-calories pellets based on body weight as an *ad libitum* fed group) have been reported to positively impact life-span and overall health of the animals.

Calorie restriction is an accepted practice for long-term housing of some species such as some rodents and rabbits, and as an adjunct to some clinical and research procedures. Animals on long-term caloric restriction regimens should be weighed at least weekly or as otherwise stipulated in the ASP. Provision of food should be recorded. As weight loss is expected with long-term caloric restriction, close monitoring of the animals is advised. Special attention should be given to ensure that the diet fed meets the animal's nutritional needs. The degree of food restriction that is clinically appropriate and humane will vary by the individual animal, its environment, and the experimental paradigm. In general, the total caloric intake of a calorie restricted animal is 10-40% less than that associated with *ad libitum* feeding.

If signs of mineral or vitamin deficiency become apparent, the veterinarian should be consulted immediately to determine appropriate treatment or supplementation to remedy the situation. The National Research Council has published several books on nutrient requirements for various species which may be a helpful resource to determine the required levels for individual nutrients needed to maintain health. [Agriculture - Animal Health and Nutrition | The National Academies Press](#)

FOOD REINFORCEMENT: Whenever an animal obtains any portion of its diet through food reinforcement, the PI must ensure that the sum of the nutritional value of all food received (either earned through behavioral paradigms or provided "free") is sufficient to maintain the animal in a healthy state. Some food

should be provided every day, unless a specific exception is described in the ASP (See note below on USDA record keeping). Experience has demonstrated that short periods, generally 48 hours or less, of markedly reduced food intake may be required during the initial phases of food regulation, or after periods of increased food intake. Furthermore, a short period (e.g., 24 hours even in the smallest species) without food intake in normal healthy animals does not typically cause adverse effects.

Note that USDA regulated species (e.g., dogs, cats, nonhuman primates, etc.) have specific requirements for water: Dogs are required to have continuous access to water unless restricted by the attending veterinarian. Other regulated species are to be offered water not less than twice daily for at least 1 hour each time. Offering water less frequently than this, should be scientifically justified, approved by the IC ACUC, and included in the annual report to USDA as an exception to the standards and regulations under the Animal Welfare Act.

The *Guide* states, “In the case of conditioned-response research protocols, use of a highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended.” Palatable motivating foods, fluids, or other desirable rewards should be used instead of restriction or to minimize restriction whenever possible. Some invariant degree of dietary control is most often required to provide consistent motivation for reliable control over the experimental behavior, though specifics will depend on the species, behavioral task, and requirements of the research design. Control parameters associated with limited daily feeding (e.g., 1 hour per day access to food) should be described within the ASP.

TARGETED WEIGHT REDUCTION: The maximum planned weight reduction should be stipulated in the ASP and the plan for achieving the targeted weight reduction should be designed to maintain the animal’s well-being throughout the process. A 15% reduction in body weight is a common goal in food regulation in rodents. When the targeted weight reduction is greater than 15% of the animal’s starting body weight, the rationale for a greater than 15% weight reduction should be scientifically justified within the approved ASP.

The complexity of the task to be trained, number of trials to be completed, the animal’s abilities and the scientific objectives of the study must be carefully considered in light of the amount of weight reduction that is needed. An animal completing a low number of easy tasks will likely not require 15% weight reduction in order to be motivated to participate; however, an animal required to perform a very complex task many times may well require this amount of weight loss to ensure consistent motivation even as the task becomes more challenging. In any case, steps to reduce anxiety in handling longer training, desensitization, and behavioral acclimation before testing or the use of highly valued rewards should all be considered as alternatives to greater weight reduction. While gradual weight reduction appears to induce overall low risk to health, animals with a greater than 15% weight loss under dietary control may be more susceptible to the deleterious effects of a short-term fast or other stressors and may require closer monitoring than other animals. It is recommended that animals be gradually reduced to a target weight and acclimated to the feeding schedule to mitigate the stress response. Ideally in rodents, the degree of diet restriction should be limited so that the initial body weight is not reduced by more than 10% during the first week. There is guidance to assist in determining study endpoints as it relates to weight loss (ARAC [Guidelines for Endpoints in Animal Study Proposals](#)).

RECORDKEEPING: According to the *Guide*, animal weights should be recorded, and weight records kept for all animals undergoing food regulation and water control. Weight records must be updated a minimum of once each week during food regulation and should be available for examination by the Facility Veterinarian and the ACUC. Animals undergoing water control may require more frequent weighing and

weight documentation than once a week depending on the water needs of the particular species involved. A daily record of the food earned during the testing session and any supplemental food provided to the animal may be required. In the case of chronic food restriction, the use of species, age, and strain-specific target growth rates is more appropriate than using a fraction of age-matched free-fed animal weights as a target. Where these data are not available, if an animal shows a loss in body weight of more than 15% during the period of study, when compared to the animal's pre-study unregulated weight, the animal should be evaluated in consultation with a veterinarian. If an increase in the amount of food provided or frequency of provision is clinically indicated, the amount of food provided, or frequency of its provision must be increased unless scientifically justified not to do so.

CLINICAL CONSIDERATIONS: The maximum percentage of body weight loss while an animal is on food regulation should not exceed 20% of its initial body weight. *Exceptions may be approved by the ACUC if scientifically justified and/or if the Attending or Facility Veterinarian determines that the weight loss does not endanger the animal's health.* Examples of exceptions may include a) species that experience seasonal fluctuations in body weight; b) obese animals that are placed on caloric restriction; or c) animals that briefly exceed their 15% weight loss target. When evaluating an animal with a 15% weight loss that was previously obese, the veterinarian may establish a body weight for an individual animal that is closer to its "ideal" weight. In such situations, the animal's clinical record must clearly indicate the weight that should be used rather than their pre-food regulated weight for future 15% weight loss assessments.

Physical evaluation of the animal by a veterinarian may include verification of changes in palpable muscle mass and body condition scores. Urinalysis and evaluation of serum chemistry (e.g., serum protein, albumin levels, etc.) can be helpful for assessing clinical health in animals under dietary control. Pale eye color in albino rodents or low hematocrit may be attributable to nutritional deficiency anemia. Low blood total solids identified by refractometry may be attributable to 'protein' deficiency. In addition, it may at times be helpful to monitor an animal for signs of ketosis or metabolic acidosis.

Animals on food regulation should be allowed a short-term unrestricted feeding period prior to any surgical procedure to avoid the development of hypoglycemia during the recovery period.

Rodents with clinical evidence of malocclusion should not be entered on studies involving control of food or fluid consumption. Clinical signs resulting from subclinical malocclusion in rodents may be exacerbated during periods of food regulation and necessitate their removal from study.

RETURN TO AD LIBITUM FEEDING: In some long-term research designs involving food regulation, the experimental design of the ASP may include intermittently allowing animals a period of *ad libitum* feeding sufficient to establish a new unrestricted feeding body weight plateau. This may be necessary if the animal stops performing, or the veterinarian determines that the animal's current weight endangers its health. When transitioning an animal from a controlled food access paradigm to *ad libitum* access, careful monitoring of the animal's dietary intake may be recommended to aid in the prevention of deleterious gastrointestinal complications (e.g., "bloat" in primates and dogs, a condition in which gastric distension can become life threatening). If animals are subsequently placed on diet restriction, the new unrestricted feeding weight may be less than the previous one, and a physical exam and serum chemistry evaluation is advisable.

Fluid Regulation:

FLUID REINFORCEMENT: As with food intake, whenever an animal obtains any portion of its fluid requirements through fluid reinforcers in behavioral testing, the PI must ensure that the sum of the fluid

earned through reinforcement and the fluid provided outside of the experiment is sufficient to maintain the animal in a healthy state. The nature (e.g., water, fruit juice) and, if applicable, concentration of the fluid reinforcement should be specified in the ASP.

Experience has demonstrated that the transition of an animal to a controlled water access paradigm is best accomplished through a gradual, systematic limitation of fluid intake over a several-day period. If possible and consistent with the experimental paradigm, concurrent with the systematic limitation of available free-choice water, animals should be provided with an opportunity to work for additional water until satiated or given water ad libitum for a brief period after the session. In some cases, the regulation can be relaxed or reduced after the animal becomes proficient at a given task. However, as with control of food intake, some invariant degree of fluid regulation is most often required to provide consistent motivation to ensure reliable control over the experimental behavior.

Experience has demonstrated that short periods with markedly reduced fluid intake may be required during the initial phases of a research design requiring water control to provide motivation for drinking, tasting tests, etc. The duration of the period will vary with the species and hydration status of the animal. Many, larger species of nonhuman primates do well with markedly reduced fluid intake for short periods, but smaller species, especially some New World species (e.g., squirrel monkeys and marmosets), may be especially susceptible to the effects of fluid restriction. Experience has demonstrated no adverse consequences of short periods (<24 hours) of reduced fluid intake in normal, healthy animals. However, consideration must be given for the species/strain being used and the animal's size, age, health status, body condition and concurrent testing and/or treatments. If a period with markedly reduced fluid intake is required after initial induction or training phases, the PI should provide clear justification as well as the extent and duration of fluid reduction in his or her ASP. There is guidance to help in determining endpoints (ARAC [Guidelines for Endpoints in Animal Study Proposals](#)).

RECORD KEEPING: As per the *Guide*, records should be maintained as appropriate for the fluid control paradigm. For example, during acute water restriction, e.g., where water is available for a minimum of fifteen minutes each day for a period of a few days with ad libitum food, a daily record of fluid access is necessary, but a record of body weight may not be necessary. However, paradigms that require repeated periods of acute water restriction or chronic controlled fluid access may necessitate more stringent recordkeeping such as a periodical record of body weight and a daily record of the volume of fluid earned during the testing session and any supplemental fluid and/or fruit provided to each animal. Consideration may be given to recording rodents' bodyweight twice a week.

Note that USDA regulated species (e.g., dogs, cats, nonhuman primates, etc.) have specific requirements for water: Dogs are required to have continuous access to water unless restricted by the attending veterinarian. Other regulated species are to be offered water not less than twice daily for at least 1 hour each time. Offering water less frequently than this, should be scientifically justified, approved by the IC ACUC, and included in the annual report to USDA as an exception to the standards and regulations under the Animal Welfare Act.

CLINICAL CONSIDERATIONS: Most animals tolerate acute water restriction for as long as 24 hours without clinically overt signs of physiologic distress or behavioral abnormalities. Normal physiological responses to fluid control routinely result in robust changes in the animal's clinical pathological status. For example, acute fluid control will often result in elevated clinical parameters (e.g., Hematocrit, Serum Total Protein, BUN, etc.), while physical and behavioral assessment of the animal indicates that the animal is healthy and adapting normally to the controlled access paradigm.

Even though animals may learn to work in a manner that earns their entire daily fluid requirement during the testing session, consideration must be given to monitoring and intervention criteria to maintain the health of the animals and avoid any detrimental effects resulting from fluid control. If at any time assessment criteria indicate that an animal needs intervention, the veterinarian in consultation with the investigator will reassess the previously established monitoring and intervention criteria for the controlled fluid paradigm and amend the ASP as appropriate.

It is recommended that at the start of a new study group, the amount of fluid consumed, body weight and hydration assessment be recorded daily for each animal to obtain a baseline prior to experimental manipulation. Once baseline fluid intake has been established, each animal should be allowed to earn fluids to satiety or to a level approved in the ASP. As needed, fluid intake should be appropriately supplemented each day. In cases in which supplements are required, the minimum amount of fluids to be provided each day should be equivalent to the amount typically consumed by the animal when it is permitted to earn fluids to satiety or to the level stipulated in the approved ASP. It is recognized, however, that to ensure the animal's welfare and experimental integrity, daily adjustments in fluid intake are often required during the research. Once an animal has learned a behavior, the daily amount of fluid provided should be increased to the maximum level that will ensure adequate and reliable performance of the task.

Some animals on a chronic controlled fluid access paradigm may decrease their total caloric intake in response to changes in their access to water. Because food intake is correlated to the amount of fluid consumed, monitoring food consumption in addition to body weight can be a valuable tool. In most cases, the decreased caloric intake is minor and does not result in a body weight loss greater than 15%. However, in the case of obese animals or those experiencing chronic fluid deficiency, loss of body weight more than 15% has been observed. This weight loss does not pose a problem in the case of obese individuals but can lead to severe complications in the case of a chronic fluid deficiency. Therefore, as a precaution against the development of clinical signs of morbidity during a chronic controlled fluid access paradigm, the animal's weight must be measured and recorded no less often than weekly. If an animal shows a loss in body weight of more than 15% during the period of study, when compared to the pre-study weight of the animal, the animal must be evaluated by a veterinarian and, if required, fluids and/or food increased appropriately. Exceptions are allowed only if the Attending or Facility Veterinarian determines that an animal is adequately hydrated and that the weight loss or other clinical signs do not endanger the animal's health or there is a previously approved and scientifically justified exception stipulated within the ASP such as may be approved for obese animals which are placed on fluid restriction. When evaluating a previously obese animal with a 15% weight loss, the veterinarian may establish an "ideal" weight for the animal. In such situations, the veterinarian must clearly indicate in the animal's permanent medical record that the "ideal" weight is to be used for future 15% weight loss assessments rather than the pre-fluid control weight.

RETURN TO AD LIBITUM FLUID: "Vacations" from controlled fluid intake paradigms may be described in some ASPs. A "vacation" is a period, ranging from a day to a few weeks, where the animal is provided either unrestricted or markedly increased fluid allocation, commonly >1.5-3 times their routine daily consumption. Following a "vacation" period, an animal may require a period without fluid intake to regain the motivation to perform their learned task. The PI must convey within the ASP for consideration by the ACUC how the benefits of a "vacation" period outweigh any subsequent requirement for marked fluid restriction.

In studies utilizing water as a reinforcer over several weeks or longer, when experimental sessions involving that reinforcement are to cease, the plan for gradually increasing the animal's consumption to ad libitum access should be outlined in the ASP. In addition, it is recommended that animals be provided with access to fluid for some period on days when research procedures are not scheduled, unless scientifically justifiable reasons preclude such fluid supplementation. As for controlled food access, when transitioning an animal from a controlled water paradigm to ad libitum fluid access, careful monitoring of the animal's dietary intake may be recommended to aid in the prevention of deleterious complications.

Summary:

It is imperative that investigators, animal care staff, and veterinarians working with animals on food or fluid controlled access paradigms know the species-typical signs of distress for the animals with which they are working (See ARAC [Guidelines for Endpoints in Animal Study Proposals](#)). Animals routinely adapt well to the research design and display no signs of distress. Animals must be carefully monitored daily to ensure that they are healthy, adapting normally, and consume sufficient food and/or water to maintain good health. Close monitoring is particularly important when an animal is initially acclimated to food or water control, during transition back to an *ad libitum* state or when increasing the difficulty of the behavioral task. In all situations, the details of the training paradigm used, monitoring and intervention criteria, and accountability of the individuals involved must be clearly outlined in the approved ASP. In addition, ACUCs should carefully evaluate the necessary level of food/fluid restriction or controlled access, whether any supplements will/should be used, what is the plan to ensure the animal's physiological and psychological well-being, what parameters will be used to monitor health and well-being, what is the intervention plan for animals failing to meet established health parameters, and whether a longer acclimation period, improved training methods, or higher value rewards could be used to reduce the need to restrict food/fluid provided.

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