

## Guidelines for Diet Control in Behavioral Studies

Behavioral research often requires that an animal perform a task for which it receives a food or fluid reward.<sup>1, 2, 5</sup> This situation resembles conditions in the wild, in which animals must forage, travel extended distances, solve problems, or otherwise work to obtain their food and water. In the professional judgment of many investigators, veterinarians, and animal behaviorists, performing a task for rewards is behaviorally enriching for laboratory animals. As noted in the *Guide*, “the least restriction that will achieve the scientific objective should be used”.<sup>8</sup> In the development of protocols utilizing food or water regulation, investigators must address three fundamental issues: a) the necessary level of regulation; b) the potentially adverse consequences of regulation; and c) the methods for assessing the health and well-being of their animals. Consideration of these three issues can facilitate the establishment of interventional endpoints to maintain the health and well-being of the animals under study.<sup>6</sup> Each ACUC must evaluate the pain-distress categorization of animals that are on restricted diets in accordance with USDA Animal and Plant Health Inspection Service, Animal Care Policy #11 (<http://www.aphis.usda.gov/ac/policy/policy11.pdf>). It is recommended that experimental animals on food or fluid regulation be weighed several times a week, ideally before experimental sessions.<sup>7</sup>

Young, developing animals have additional dietary requirements for maintaining their normal rate of growth. Investigators working with young animals should specifically address, in their animal study proposal, their expectation for any retardation of growth rate and adult size. Comparisons with litter mates or other similar control animals may prove useful when assessing growth in young, developing animals. In many situations, when caloric or fluid restriction has been justified in developing animals, animals may never reach their projected adult size, but will in all other respects develop into normal adults. In all situations, young, developing animals on restricted food or fluid regimens should be carefully monitored by investigative, veterinary and husbandry personnel.

In all situations, professional judgment must be used to ensure the well-being of the animal throughout the period of study. Consideration must be given for the species, strain, body condition, and/or hydration status of each animal. The purpose of this document is to provide investigators with guidelines for the proper use of diet control in behavioral studies in which food or fluid consumption is not the independent variable in the research design.

### **Food**

Whenever an animal obtains any portion of its diet through food reward, the investigator must ensure that the sum of the nutritional value of the food earned through reward and of the food provided "free" (without the necessity of earning it) is sufficient to maintain the animal in a healthy state. Whenever possible, the food reward should be a substance that is sufficiently rewarding and motivating (e.g., raisins, peanuts) such that dietary restriction is minimized or unnecessary. However, dietary control is often required to provide motivation for performing a task, depending on the species, the behavioral task, and the requirements of the research design.<sup>1, 3</sup> In such cases, some food should be provided every day, unless a specific exception to this policy has been obtained in an approved animal study proposal. Experience has demonstrated that short periods, generally forty-eight hours or less, of markedly reduced food intake or fasting may be required during the initial phases of diet control, or after periods of increased food intake (e.g., ad libitum food availability). To date, experience has demonstrated no adverse consequences to a short period without food intake in normal

healthy animals. However, if any period of markedly reduced food intake or fasting is required, the principal investigator should provide a clear justification for the reduced food intake, as well as the extent and duration of food reduction in his or her animal study proposal.

When caloric control is an experimental requirement, other aspects of the animals' diet should remain balanced (e.g., vitamins, minerals, etc.). Weight records must be kept for all animals on dietary control, a minimum of once each week, and should be available for examination by the veterinary staff and the institute Animal Care and Use Committee.

An animal's weight must be measured and recorded at no less than weekly intervals. If an animal shows a loss in body weight of more than 15% during the period of study, when compared to the highest recorded weight of the animal, the animal must be evaluated by a veterinarian and, if required, its food increased appropriately. One exception to the above rule is an obese animal that is placed on caloric restriction. When evaluating an animal with a 15% weight loss that was previously obese, the veterinarian may determine that the current weight of the animal is closer to the "ideal" weight for the animal. In such situations, the veterinarian must clearly indicate in the animal's permanent medical record that the animal's current weight is used rather than their highest recorded weight for future 15% weight loss assessments. Exceptions to this policy are allowed only if the attending or facility veterinarian determines that the weight loss does not endanger the animal's health.

Many investigators have maintained normal healthy animals with a 15% weight loss or more. Therefore, there appears to be a low risk to the animal's health in using the 15% weight-reduction limit. However animals with a 15% weight loss under dietary control maybe more susceptible to the deleterious effects of a short-term fast and may require closer monitoring than other animals. Animals on diet control should be allowed a short-term unrestricted feeding period prior to any surgical procedure to avoid the development of hypoglycemia during the recovery period.

It is recommended that animals be gradually reduced to a target weight and acclimated to the feeding schedule to mitigate the stress response.<sup>6</sup> Ideally, the diet restriction should be limited so that the body weight is reduced not more than 10% per week.

Special attention should be given to ensure that the diet fed meets the animal's nutritional needs.<sup>6</sup> In general, the total caloric intake of a food-regulated animal is 50-70% of that associated with ad libitum feeding.<sup>6,9</sup> With the exception of short term fasts, it is recommended that animals should be fed a daily ration containing at least 30% of their minimum caloric requirements. Physical evaluation of the animal by a veterinarian, changes in palpable muscle mass and evaluation of serum chemistry (e.g., serum protein, albumin levels, etc.) can be helpful for assessing clinical health in animals under dietary control. In addition, it may at times be helpful to monitor an animal for signs of ketosis or metabolic acidosis.

It may be advisable in some long-term research designs (often termed chronic preparations) to intermittently allow animals a sufficient period of ad libitum feeding to establish a new plateau of unrestricted body weight after a period of diet control. This may be necessary if the attending or facility 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 is recommended to aid in the prevention of deleterious gastrointestinal complications (e.g., "bloat" in primates, a condition in which gastric distension can become life threatening).

### **Fluid**

As with food intake, whenever an animal obtains any portion of its fluid requirements through fluid rewards in behavioral testing, the investigator must ensure that the sum of the fluid earned through reward and the supplemental fluid provided outside of the experiment is sufficient to maintain the animal in a healthy state. 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.<sup>1</sup> Whenever possible, concurrent with the systematic limitation of available free-choice water, animals should be provided with an opportunity to work for additional water until satiated. In many cases, the restriction often may be relaxed or reduced after the animal becomes proficient at a given task.<sup>6,7</sup> It is recommended that at the start of a new research protocol the amount of fluid consumed, body weight and hydration assessment be recorded daily for each animal.<sup>6</sup> Some animals on controlled fluid paradigms are provided with "vacations". A "vacation" is a period of time, ranging from a day to a few weeks in duration, when the animal is provided a markedly increased fluid allocation, commonly >1.5-3 times their routine daily consumption. When an animal is not required to perform their learned task for prolonged periods of time, several weeks duration or longer, gradually increasing the animal's consumption to ad libitum access is recommended. In addition, it is recommended that animals be provided with free access to fluid for some period on days when research procedures are not scheduled, unless scientifically justifiable reasons preclude such fluid supplementation.<sup>7</sup> When transitioning an animal from a controlled water paradigm to ad libitum fluid access, careful monitoring of the animal's dietary intake is recommended to aid in the prevention of deleterious gastrointestinal complications (e.g., "bloat" in primates, as described above for food control).

Experience has demonstrated that short periods without or with markedly reduced fluid intake may be required during the initial phases of a research design requiring water control. 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 or no fluid intake for periods up to thirty-six hours, but smaller species, especially some New World species, may be more susceptible to the effects of marked fluid restriction. Similarly, following a "vacation" period, an animal may require a period without fluid intake to regain the motivation to perform their learned task. To date, experience has demonstrated no adverse consequences of short periods without fluid intake in normal, healthy animals.<sup>4</sup> However, if any period without or with markedly reduced fluid intake is required, the principal investigator should provide a clear justification for the reduced fluid intake, as well as the extent and duration of fluid reduction in his or her animal study proposal.

Because the difficulty of a given behavioral task can, in part, determine the degree of water control required, whenever possible animals should be acclimated to easy task(s) prior to their systematic and gradual progression to more difficult tasks. Once a baseline fluid intake has been established on a given task, each animal should be allowed to earn fluids to satiety or its fluid intake should be appropriately supplemented on a daily basis. 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. It is recognized, however, that to ensure the animal's welfare and experimental

integrity, daily adjustments in fluid intake are often required during the course of 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 performance of the task.

### **Assessment of Adequacy of Fluid Intake**

Even though animals typically learn to work in a manner that earns their entire daily fluid requirement during the testing session, a number of precautions must be taken to avoid the detrimental effects of fluid control. The nature (e.g., water, fruit juice) and, if applicable, concentration of the fluid reward should be specified in the animal study proposal. Daily records of fluid intake must be maintained and be available for review by the veterinary staff and the institutional ACUC. The daily record should indicate the fluid earned during the recording session and any supplemental fluid and/or fruit provided to the animal. Each animal under fluid control must be observed daily for its health status by the animal care staff. Normal physiological responses to fluid control routinely result in changes in the animal's clinical pathological status. For example, fluid control will often result in elevated blood parameters (e.g., Hematocrit, Serum Total Protein, etc.), while physical and behavioral assessment of the animal indicates that the animal is healthy and adapting normally to the controlled access paradigm.<sup>1,2</sup> If at any time the attending veterinarian determines that an animal is not adapting sufficiently to the controlled fluid paradigm, the veterinarian will consult with the investigator to develop a plan to maintain the health of the animal.

Some animals on a 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 can also be a valuable tool. In most cases, the decreased caloric intake is minor and does not result in a body weight loss greater than fifteen percent (>15%). However, in the case of obese animals or those experiencing chronic fluid deficiency, loss of body weight in excess of 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 chronic fluid deficiency, the animal's weight must be measured and recorded at no less than weekly intervals. If an animal shows a loss in body weight of more than 15% during the period of study, when compared to the highest recorded weight of the animal, the animal must be evaluated by a veterinarian and, if required, its fluids increased appropriately. One exception to the above rule is an obese animal which is placed on fluid restriction. When evaluating an animal with a 15% weight loss which was previously obese, the veterinarian may determine that the current weight of the animal is closer to the "ideal" weight for the animal. In such situations, the veterinarian must clearly indicate in the animal's permanent medical record that the animal's current weight be used rather than their highest recorded weight for future 15% weight loss assessments. Exceptions to this policy are only allowed only if the attending or facility veterinarian determines that an animal is adequately hydrated and that the weight loss does not endanger the animal's health.

### **Summary**

It is imperative that investigators, animal care staff and veterinarians working with animals on food or water controlled access paradigms know the species-typical signs of distress for the animals with which they are working. Animals routinely adapt well to the research design and display no signs of distress.<sup>1</sup> Animals must be carefully monitored on a daily basis 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 lib state or when increasing the difficulty of the behavioral task. In all situations, the details of the training paradigm used and accountability of the individuals involved must be clearly outlined in the approved animal study proposal. Experience has demonstrated that diligent record keeping on the daily food or fluid volume consumed, hydration status, appearance, general affect, experimental performance, and routine weighing are reliable for the identification of changes in behavior patterns. Records should be reviewed regularly and kept accessible to veterinary staff and others who have a need to evaluate them. The daily records should indicate the food or fluid earned during the recording session and any supplemental food or fluid provided to the animal. In addition, a plan of action, complete with endpoints for therapeutic intervention, should be considered when the experimental animal proposal is developed.<sup>6</sup>

## References

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