

# **Guidelines for Diet Control in Behavioral Animal Studies**

## **Introduction**

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

Behavioral research often requires that an animal perform a task for which it receives a food or fluid reinforcement.<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 reinforcement is behaviorally enriching for laboratory animals. As noted in the Guide, "the least restriction that will achieve the scientific objective while maintaining animal well being should be used."<sup>8</sup> In the development of protocols<sup>16,17</sup> utilizing food or water control, investigators must address three fundamental issues: a) the necessary level of control; b) the potentially adverse consequences of control; and c) the methods for assessing the health and well-being of their animals. In addition, the species being used and the animal's size, age, health status and concurrent treatments must also be considered. Consideration of these factors can facilitate the establishment of interventional endpoints to maintain the health and well-being of the animals under study.<sup>6</sup>

It is recommended that experimental animals on food or fluid control be weighed several times a week, ideally before experimental sessions.<sup>7</sup> At a minimum, body weights should be recorded at least weekly and more often for animals requiring greater restrictions.<sup>6</sup> Written records should be maintained for each animal to document daily food and fluid consumption, hydration status and any behavioral and clinical changes which are to be used as criteria for the temporary or permanent removal of an animal from study.<sup>8</sup> Each ACUC must evaluate the pain-distress categorization of animals that experience diet control in accordance with USDA Animal and Plant Health Inspection Service, Animal Care Policy #11

([http://www.aphis.usda.gov/animal\\_welfare/downloads/policy/Policy%2011%20Final.pdf](http://www.aphis.usda.gov/animal_welfare/downloads/policy/Policy%2011%20Final.pdf)).

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, with similar control animals, or with growth standards will prove useful when assessing growth in young, developing animals, and in many genetically modified animal models. 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.

## **Food**

Whenever an animal obtains any portion of its diet through food reinforcement, the investigator must ensure that the sum of the nutritional value of the food earned through reinforcement and of the food provided "free" (without the necessity of earning it) is sufficient to maintain the animal in a

healthy state. When possible, the food reinforcement should be a substance and size that is sufficiently reinforcing and motivating (e.g. through the use of well-defined pellets containing a uniform sugar or high-fat component that are commercially prepared for reinforcement procedures) such that dietary restriction is minimized. Some invariant degree of dietary control is most often required to provide consistent motivation for reliable control over the experimental behavior, though specifics may, depend on the species, the behavioral task, and the requirements of the research design.<sup>1,3</sup>

Though ad lib feeding is the normal practice in the vivarium, ad lib feeding is not the norm for rodents in the wild. Many standard control rats and mice used in biomedical research are sedentary, obese, glucose intolerant, and on a trajectory to premature death.<sup>14</sup> Three dietary restriction protocols have been reported to positively impact life-span and overall health of the animals: every-other-day feeding (unlimited access to regular food pellets, on an alternate day feeding schedule); paired feeding (equal amounts of reduced-calories pellets by weight as the ad libitum group) and limited daily feeding (access to food for a limited time window of 1 hour per day). 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 surgical procedures<sup>8</sup>. Animals on long term caloric restriction regimens should be weighed at least weekly, and observed daily for signs of distress. As weight loss is expected with long term caloric restriction, close monitoring of the animals by the veterinarian, and periodical assessment of clinical health (e.g., serum chemistry) is advised.

In most cases, some food should be provided every day, unless a specific exception is described 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). Experience has demonstrated no adverse consequences to a short period (e.g. 24 hours even in the smallest species) without food intake in normal healthy animals.<sup>13</sup> In contrast to ad lib feeding, long term diet control has been shown to greatly enhance longevity and significantly improve animal health and well-being. Brief periods of fasting of 24 hours or less, preceded by ad lib feeding of at least forty-eight hours is not considered to be diet control and does not require the recording of the animal's weight.

Consideration must be given for the species being used and the animal's size, age, health status, body condition and concurrent treatments. 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. 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.

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 may be 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.

When caloric control is an experimental requirement, other aspects of the animals' diet should remain balanced (e.g., vitamins, minerals, etc.). Animals' weights must be recorded and weight

records must be kept for all animals on dietary control. Weight records must be updated a minimum of once each week, and should be available for examination by the veterinary staff and the institute Animal Care and Use Committee. 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.<sup>13</sup> 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 pre-diet control weight of the animal, the animal must be evaluated in consultation with a veterinarian and, if deemed necessary, its food increased appropriately unless scientifically justified. The maximum percentage of body weight loss while an animal is on diet control should not exceed 20% of its initial body weight.<sup>15</sup> Exceptions are allowed if scientifically justified and/or if the attending or facility veterinarian determines that the weight loss does not endanger the animal's health, e.g. for species that experience seasonal fluctuations in body weight. One example of an exception to the above rule involves obese animals that are placed on caloric restriction. When evaluating an animal with a 15% weight loss that was previously obese, the veterinarian may determine a weight of the animal that is closer to its "ideal" weight for the animal. In such situations, the animal's permanent record must clearly indicate the weight that should be used rather than their pre-diet control weight for future 15% weight loss assessments. 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. 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 diet control and necessitate their removal from study. Pale eye color in albino rodents or low hematocrit may be attributable to nutritional deficiency anemia. Low blood total solids 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. 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 when establishing the target weight.

It may be useful in some long-term research designs involving diet control (often termed chronic preparations) to intermittently allow 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 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 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 and clinical exam is advisable. It is noteworthy that long-term calorie restriction, at up to 40% restriction in caloric intake without changes in any other dietary component, significantly extends life span in several animal models, including mice, rats and primates.<sup>10,11</sup> This dietary manipulation also greatly reduces the incidence of age-related cancers and age-associated neurocognitive impairment.<sup>12</sup> The cognitive abilities of rats maintained on restricted feeding schedules or provided access to a running wheel are superior to rats maintained under standard housing conditions.<sup>14</sup>

## **Fluid**

As with food intake, whenever an animal obtains any portion of its fluid requirements through fluid reinforcers in behavioral testing, the investigator must ensure that the sum of the fluid earned through reinforcement 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> 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. In some cases, the restriction often can be relaxed or reduced after the animal becomes proficient at a given task.<sup>6,7</sup> However as with control of food intake, some invariant degree of fluid restriction is most often required to provide consistent motivation ensuring reliable control over the experimental behavior.

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 (36) hours, but smaller species, especially some New World species, (such as squirrel monkeys), may be especially susceptible to the effects of fluid restriction. Experience has demonstrated no adverse consequences of short periods without fluid intake in normal, healthy animals.<sup>4,13,15</sup> However, consideration must be given for the species being used and the animal's size, age, health status, body condition and concurrent testing and/or treatments. If any period without or with markedly reduced fluid intake is required after initial phases, 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.

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> Once a baseline fluid intake has been established on a given task, each animal should be allowed to earn fluids to satiety or to a level approved in the Animal Study Proposal. As needed, 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 or to the level approved in the Animal Study Proposal. 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 and reliable performance of the task.

"Vacations" from controlled fluid intake paradigms may be advisable in some protocols. A "vacation" is a period of time, ranging from a day to a few weeks in duration, when the animal is provided unrestricted or a markedly increased fluid allocation, commonly >1.5-3 times their routine daily consumption. In studies utilizing water as a reinforcer, during periods of several weeks duration or longer, when experimental sessions involving that reinforcement are suspended, gradually increasing the animal's consumption to ad libitum access is recommended. In addition, it is recommended that animals be provided with additional 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). Following a "vacation" period, an animal may require a period without fluid intake to regain the motivation to perform their learned task. The ACUC must balance the benefits of a "vacation" period with the subsequent need for marked fluid restriction.

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

Mice and rats may be acutely water deprived for as long as 24 hours to provide motivation for drinking or tasting tests. Mice and rats tolerate this without clinically overt signs of physiologic distress or behavioral abnormalities. Each animal under fluid control must be observed daily for its health status by the animal care or investigative staff. Normal physiological responses to fluid control routinely result in robust 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>1,2,22</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.

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 reinforcement 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 testing session and any supplemental fluid and/or fruit provided to the animal. Therefore, during acute water deprivation, if water is available for a minimum of fifteen minutes per day with ad lib food, body weights records are not required.<sup>15</sup> Similarly, food and water deprivation for periods of 24 hours or less preceded by ad lib food and water for at least 48 hours do not require body weight records. However, paradigms that require repeated periods of acute water deprivation over several weeks to months should be considered to be a chronic controlled fluid access paradigm.

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 fifteen percent. 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 during a chronic controlled access paradigm, 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 pre-diet control weight of the animal, the animal must be evaluated by a veterinarian and, if required, its fluids 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 does not endanger the animal's health or there is a scientific justification.

One such exception to the above rule involves obese<sup>18</sup> animals which are placed on fluid restriction. When evaluating a previously obese animal with a 15% weight loss, the veterinarian may determine a weight of the animal that is closer to its “ideal” weight for the animal. In such situations, the veterinarian must clearly indicate in the animal’s permanent medical record the weight to be used rather than their pre-diet control weight for future 15% weight loss assessments.

### **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 libitum 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 ensuring that subjects remain in a healthy state. Records should be maintained as appropriate, reviewed regularly, and accessible when necessary. 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 Study Proposal is developed.<sup>6</sup>

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