

Association of Primate Veterinarians Food Restriction Guidelines for Nonhuman Primates in Biomedical Research

The Association of Primate Veterinarians has developed these guidelines to provide information on how to manage food restriction protocols that may be used for research experiments to modulate motivation in nonhuman primates (NHPs). It is common for animals on these types of studies to be motivated to participate through the use of food restriction. Restriction in this document refers to either limiting the time the animal has access to food (temporal restriction) or limiting the amount of food that the animal has access to (caloric restriction). Either way, hunger is the motivator for performance. These guidelines will address considerations when performing either of these techniques for food restriction but are not applicable to nutritional studies whose primary goal is to study the effects of various degrees of food restriction. Nor are these guidelines meant to address food restriction in evaluated obese animals under veterinary care for weight management.

Considerations for Using Food Restriction as a Motivator

Limited access to food and having to work for food is not a foreign concept or inherently detrimental to NHPs.¹ In their native environments it is common to have limited access to certain foods during certain seasons or times of day. It is well established that foraging and feeding is an important and large part of how NHPs, in particular macaques, spend their waking hours. It is recommended in both the USDA regulations and the *Guide for the Care and Use of Laboratory Animals* that NHPs be provided an opportunity to work for their food through foraging and/or engaging with enrichment devices such as puzzle feeders.² Studies have shown that long-term calorie restriction can significantly extend life span and greatly reduce the incidence of age-related cancers and age-associated neurocognitive impairment.^{3,4} When the primary goal of food restriction is for experimental purposes, and not for the well-being of the animal (disease management, weight management, environmental enrichment), it needs to be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Any time an animal is placed on food restriction, whether it be for health or experimental purposes, there should be a system in place for regular routine monitoring of the animal's health and well-being so that necessary adjustments can be made.

When considering to use food restriction as a motivator, researchers should consider and thoroughly discuss alternatives. The *Guide* states that "...highly preferred food or fluid as positive reinforcement, instead of restriction, is recommended".⁴ Investigators should refer to published results of successful use of highly preferred foods/treats as positive reinforcement, conditioned reinforcers, and social stimuli as motivators.^{5, 6, 7} Investigators are also encouraged to seek advice from NHP specialists (veterinarians, behaviorists, animal care staff) and experienced colleagues regarding the species of animal and specific operant behavior to be performed to better understand what to reasonably expect and if food restriction is necessary to achieve their desired goal. If possible, first begin with preferred foods in a positive reinforcement system, or other positive approaches, to determine whether they may be adequate to sustain the needed performance. If it is determined that food restriction is necessary and justified, the least restrictive diet that will achieve the experimental objective and maintain animal well-being should be used.^{1, 2, 8}

Animal Selection

Candidate animals should receive a physical examination and evaluation by a veterinarian to determine their fitness to participate in a food restriction protocol. Food restriction may be contraindicated for certain individuals especially those with health conditions that can be negatively effected by restriction (e.g. diabetes, pregnancy, diarrhea, particular food sensitivities, etc.).

It should be noted that not all animals will perform well with food restriction as a behavioral motivator. Individual animals will have different abilities to learn tasks. This must be accounted for and lack of expected performance should not be automatically attributed to a lack of motivation. The tasks should not be too complex for the age or ability of the animal as this can lead to poor performance. When available, past experience of the animal may provide insight into future performance. An article by Tulip *et al.* discusses the use of an automated system in group-housed macaques for positive reinforcement and how it can potentially be “used to pre-screen animals for suitability for behavioral neuroscience research”.⁶ Such refinements have the potential to save time and resources by screening for animals most capable of learning the tasks required to complete the study.

When beginning a food restriction protocol it is important to know the baseline normal amount of food that is required for each individual animal to maintain its weight and well-being while on study. The species, age, stage of growth and health status will factor into an animal’s baseline maintenance caloric intake. Young growing animals (typically under 6 years for female macaques and under 8 years for male macaques), obese, or underweight animals have special considerations and should be evaluated in consultation with a veterinarian prior to study.¹⁰ An individual animals’ optimum food ration for growth, development, and weight maintenance must be assessed prior to initiating food restriction. It is recommended that a growth curve be produced for each animal after a period of stable housing. An individual growth curve should take the animal’s starting weight and body condition into consideration and should follow published normograms.^{7,9,10} The stabilization period can vary from several weeks to months and will depend on whether an animal is newly received, established or still growing.

Food restriction that involves quantifying the specific amount of food items an individual animal receives does not necessarily preclude that animal from social housing. The need for strict food monitoring should be balanced with the animal’s well-being and need for social contact and interaction. Even intermittent periods of social housing are beneficial to animals and any stress associated with repeated separations is superceded by the positive effects of social housing.¹¹ Equipment such as modular caging that can provide separation during feeding times but socialization the rest of the time or automatic feeders that can scan a microchip implanted in the animal’s wrist and dispense programmed amounts of feed to individual animals provide alternatives and refinements to single housing.^{12,13}

Protocol Review and Justification

The use of food restriction as an experimental technique to modulate motivation is potentially a highly stressful procedure and the IACUC must consider whether the restriction techniques proposed have been adequately justified. For studies performed in the U.S. and receiving NIH funds, the NIH’s *Guidelines for Diet Control in Laboratory Animals* stipulates that “Each IACUC must evaluate the diet control parameters, monitoring plan, intervention criteria, and pain-distress categorization the animals will experience in accordance with USDA Animal and Plant Health Inspection Service, Animal Care Policy #11”.¹⁴

Appropriate questions to consider during the protocol review stage include:

- Is food restriction essential for the research and scientifically justified?
 - It is the investigator's responsibility to show that the level of restriction for each animal is the least amount necessary to achieve the scientific goal. Implementing further restriction for the purposes of accumulating data faster is not a sufficient justification.
- What is the schedule for food restriction and access? Will there be periods when the animal will have ad libitum access to food?
 - If animals are offered their entire daily food ration on testing days, the protocol should describe how and when food will be offered on days the animal is not tested.
- How is restriction being accomplished? Has this been considered and justified?
 - It has been shown that restriction to specific times of day (e.g. post-session feeding) versus restriction to a target weight (80-85% of free feeding/ad libitum) can produce stable performance.
- Does the investigative team have adequate previous experience with training monkeys on the task proposed?
 - If not, a plan for obtaining the necessary personnel training should be described.
- What are the endpoints for removal from testing and for removal from the study?
 - A pre-emptive plan of action for how to address interventions and endpoints should be developed and described in the protocol.
- How will the animals be monitored?
 - Post-approval monitoring plans should be developed to document and ensure compliance.

Considerations When Initiating Food Restriction Protocols

- Animals should be allowed free access to water in the home enclosure while on food restriction protocols.
- Animals should not begin testing until they are first acclimated to their new housing, eating and maintaining a stable weight, and familiar with research staff. Providing a stable environment and investing time to conduct proper preparation and training (of both personnel and animals) are fundamental for experimental progress and success.
- Vitamins and supplements may be beneficial in maintaining body condition. Consultation with the facility veterinarian and/or animal care supervisor may provide guidance for metabolic equilibrium.
- The proportion of positive reinforcers (such as fruit or other food treats) which will be allowed to contribute to full ration should be determined. For example, if nutritionally complete, 100% of positive reinforcements would contribute to a full ration vs. only 25% if non-nutritional (i.e. candy or other sugar treat) reinforcers are used.
- Preferred reinforcers should be determined for each animal and used for positive motivation in an effort to decrease the level of food restriction necessary.¹⁵ Typically during introduction and training, restriction is used to motivate the animals to learn a task. Once proficient, positive reinforcement should be used to maintain the behavior with the goal of possibly eliminating or reducing restriction.
- Animals should be evaluated on an individual basis to determine their hierarchy of preference for different food items. Holding back highly preferred food items until later in the session may encourage animals to perform additional trials after becoming satiated on lower ranking food items thus facilitating a less severe food restriction.
- Food restriction should be introduced gradually over several days to weeks. Animals need time to recognize and adjust to the new feeding schedule. Studies in mice and rats have shown that animals learn and adapt to time restrictions more readily than to limitations on the amount of food provided.¹²
- Typically, animals will consume more food if offered meals twice a day than once a day. An animal fed an entire day's ration in one feeding may become satiated prior to consuming the entire ration and be more likely to waste food, resulting in reduced overall consumption.

- Problems may arise (e.g. bloat) when abrupt changes to the diet are made such as going from more extreme restriction protocols to ad libitum access to food. Gradual return to non-restricted feed should be considered when animals are on a hiatus from study.
- It can be stressful for restricted animals to see/hear other animal receiving food when they are not being fed. Consideration should be given to offering restricted animals some food when other animals in the room are being fed.
- If an animal is not completely consuming the restricted ration, no further restriction should be instituted, and the animal should be closely monitored for signs of illness and/or behavioral changes.

Ongoing Monitoring During Food Restriction Studies

The following parameters should be monitored on a regular basis by the research team and periodically evaluated in consultation with the facility veterinarian for each individual animal:

Daily:

- The amount of food provided and the amount of food consumed at each meal.
- The amount of food reinforcements offered and consumed.
- Behavior of the animals. Any stress-related or abnormal behaviors (eg. stereotypical behaviors, self-injury, excessive fear or anxiety) should be discussed with the veterinary and/or behavioral staff.

Weekly/Biweekly

- The animal's body weight should be obtained at regular intervals, preferably weekly.¹⁴ The weight should always be obtained at the same time each day, prior to experimentation and prior to feeding and watering.¹⁴ Ideally animals should be weighed on the same scales to ensure consistent readings.

Monthly/Quarterly

- Body condition should be evaluated and scored at each physical examination.¹⁶

Semi-annually/Annually

- Clinical chemistry profiles (serum chemistry and complete blood count with differential) should be reviewed at regular intervals as determined by the veterinarian in consultation with the principal investigator (PI).
- Scales used to weigh animals should be calibrated on a regular schedule and recorded.

Inexperienced personnel may attribute poor performance during sessions to inadequate restriction but there are several variables that can factor into an animal not learning or performing desirably. Variables related to health, equipment, environment, personnel, unrelated behavioral factors, and potentially excessive restriction can all contribute to an animal's performance and should be considered and evaluated prior to making changes.

Humane Endpoints¹⁷

A veterinarian should be contacted and consulted regarding temporary or permanent removal of an animal from a food restriction regimen. An animal should be temporarily removed from a food restriction motivational regime if it has lost more than 15-20% of their projected optimal body weight, has an unsatisfactory body condition score (BCS; <2.5/5), shows significant abnormal behaviors that have not improved with intervention,

or has abnormal laboratory data.¹⁷ With approval from the veterinarian, the animal may be returned to study when improvements in body weight or behavior have been made. Some animals may be determined to be small for their weight and may maintain a body condition score of 2-2.5/5 even when on ad libitum feeding. Therefore, gathering and documenting baseline data demonstrating a stable weight history can be useful to guide individual endpoint criteria. Animals that maintain a thinner body condition may need to be evaluated by a veterinarian and determined to be fit for study as well as followed more closely using weight measurements and an appropriate pre-study determined weight loss endpoint.

Animals that develop behavioral problems (which are not a part of the intended experimental model) while on study should be assessed in consultation with the veterinary department and behavioral specialists to determine whether the behavior is related to experimentation. If the animal develops severe behavioral problems that cannot be managed with medication or behavior modification techniques, the animal should be removed from study.

Consideration should be given to permanently removing animals from a food restriction motivational regime if they continue to have significant problems in any of the above identified areas after being returned to food restriction more than twice.

Record Keeping

Records must be available for review by the IACUC, clinical veterinarian, and animal caregivers.^{1,2,8} Records must be maintained to include:

- the proposed individual full ration of food.
- the degree of restriction from full ration.
- the duration of the restriction and results of monitoring parameters such as: body weight, BCS, behavioral assessments, and laboratory data.
- the individual animal's preferred food reinforcements.
- the results of behavioral training and testing such as poor, satisfactory, or good, including the length of time required to acquire specific skills.

Reporting

The clinical veterinarians should review records regularly to ensure that animals are maintaining weight and condition. The IACUC should evaluate the animal records every 6 months or more often if there are problems or concerns. Animals that are food restricted to the degree that they lose satisfactory body condition and are removed from study should be reported as experiencing more than momentary pain or distress, and should be reported to the IACUC.

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