This appendix to IACUC Guideline 21, “Guidelines for the Use of Food or Fluid Restrictions”, provides information to be used when planning and performing fluid regulation in NHPs used for research, teaching, or testing purposes at the University of Texas at Austin. It is organized into six sections:

Section A – Background Information
Section B – Food/Fluid Regulation Procedures
Section C – Monitoring Requirements
Section D – Record Keeping
Section E – Protocol Considerations
Section F – References and Acknowledgements

Section A – Background Information

A primary reference adopted by the IACUC and used to develop this IACUC guideline is the “Association of Primate Veterinarians Guidelines for Use of Fluid Regulation for Nonhuman Primates in Biomedical Research” (henceforth referred to as “APV Guidelines”). This document has been formally reviewed and adopted by AAALAC International's Council on Accreditation as guidance for accredited units and it should be referred to if/when additional details and clarification regarding water control in primates are required. Investigators may request deviations from the procedures below by “disagreeing” to these guidelines in eProtocol and describing their scientific justification. Deviations must be reviewed and approved by the IACUC.

Section B – Food and Fluid Regulation Procedures

Food and water control must be performed in a humane, refined manner in order to be considered a procedure that does not involve significant distress, since these studies are classified by the IACUC as Pain Category C. As mentioned in these guidelines, some minor weight loss and decreased consumption may occur as animals minimize their intake and become more lean as part of the adaption process. Water must not be withheld to the point that it causes acute inappetence.

PRIOR TO REGULATION

1. Prior to placement on water restriction, an animal’s average daily unrestricted fluid consumption must be calculated by documenting daily voluntary intake over a five-business day period. This will require the use of secure water bottles and must reflect water being made available throughout the day by refilling the bottle as needed. The normal daily intake of a male macaque may meet or exceed 1,000ml.
2. Prior to initiating food restriction, an animal’s average daily unrestricted food consumption must be calculated by documenting daily voluntary intake over a five-business day period. During this time, the biscuit count will not be limited to the veterinary-calculated ration, as food should be continuously available. Alternative methods of calculating minimum daily requirements (e.g. kcal/kg/day) may be used if described in the approved protocol.

3. Prior to initiating either food or fluid restriction, the animal must be given a complete physical examination by a veterinarian, and baseline criteria recorded to include body condition scoring and clinical values when possible (electrolyte levels, serum osmolality, hematocrit and total plasma protein) obtained as part of blood sampling to include a CBC and clinical chemistry. This project-specific testing is available on a fee-for-service basis from the ARC veterinary group.

INTRODUCTION TO REGULATION

1. Food and fluid regulation must be introduced gradually through a systematic limitation of intake over several days. Food and water restricted animals may over-consume when returned to an ad lib schedule, so it is best practice to also do a transition back to ad lib food and water following restriction.

2. Each animal must be provided with the opportunity to earn food and fluid reward to satiety during each work period. Animals failing to consume their calculated daily minimum food or fluid intake during a work period must be provided with supplemental food or fluids after the training session to ensure the minimum daily intake level and hydration needs have been met.

3. Projects involving water control must be planned and managed in a dynamic fashion so that the degree of water control is routinely being re-adjusted to the minimal level that provides adequate motivation and performance based on the specifics of the animal and the research task. However, there are humane limits to protect the well-being of the primate, and increased levels of restriction must be carefully managed. A well-recognized target for maximum restriction is the provision of no less than 20ml/kg per day, a level shown sufficient to induce motivation to work while having little risk of causing acute dehydration and adverse physiologic effects. Researchers must attempt to design their studies, behavioral tasks and animal assignments to accomplish their goals with this level of restriction.

   a. Restriction to allocations lower than 20 ml/kg/day should be rare and are subject to special conditions. Although it has been shown that water allocations in the range of 14-17 ml/kg/day can be tolerated when used in a five-day “workweek” with increased water on the weekend (Gray, 2016), special monitoring must be performed when water intake levels below 20 ml/kg/day are utilized. An example of a situation where a transient lower level might be required is for a brief period when NHPs are initially trained on a task, such as 15 ml/kg for 24-72 hours, after which the NHP must receive the volume withheld in addition to the 20 ml/kg/day minimum such that the total weekly water ration is unchanged. This can only be done under close supervision by both the research team and ARC personnel.

4. Projects involving food control must be managed such that the degree of food restriction is routinely being re-adjusted to the minimal level that provides adequate motivation and performance based on the specifics of the animal and the research task. In general, the total caloric intake of a food-regulated animal is 50-70% of that associated with ad libitum feeding. Therefore, food restricted animals must be given at least 50% of their ad lib intake daily. Researchers must attempt to design their studies, behavioral tasks and animal assignments to accomplish their goals with this level of restriction.
DURING THE REGULATION

1. Once an animal has learned any required task, it should be given opportunities to complete the task with less food or fluid regulation. This is commonly done by increasing the reward provided for each successful task and/or providing rewards such as juice.

2. Animals must receive increased food and fluid allotment such that they are transitioned to ad lib food and water by 48 hours prior to a surgical procedure. Increased food and fluids must continue to be provided for at least one week post-operatively (with consideration to the nature of the procedure, analgesics, antibiotics, or anti-inflammatory agents used to treat the animal). If ad libitum food/water access isn’t to be provided for the duration of this period, allotments must be increased to at least 80ml/kg/day for fluid, or 80% ad lib calories. Should urgent situations arise in which an animal must undergo general anesthesia before they can be transitioned to ad lib food or water, the ARC vet staff should be consulted to determine how to appropriately address the situation.

3. Vacations from regulated food and fluid intake should be described in the IACUC protocol. The vacation is a period of time, ranging from a day to a few weeks in duration, when the animal is provided with unrestricted or a markedly increased food or fluids (commonly 80% ad lib calories, or >1.5 to 3 times the regulated daily fluid intake). In addition, supplemental access to food or fluid on days when research procedures are not scheduled should be considered unless scientifically justifiable reasons preclude this practice.

4. Animals that become dehydrated or that physically or mentally deteriorate as a result of food or fluid regulation must be removed from study. This removal may be temporary if treatment is available or permanent if the condition is declared untreatable. This assessment must be made in consultation with an ARC veterinarian.

5. Only trained personnel should monitor and document body weight, body condition, food consumption, hydration status, and behavioral changes of the animal. Training on these parameters should be documented on the personnel training records (link)

Section C – Monitoring Requirements

This section describes requirements and includes parameters that must be monitored and documented during fluid regulations.

1. A physical examination by a veterinarian with attention to the animal’s body condition and assessment of appropriate clinical chemistry parameters must be performed prior to initiation of restriction, and at least every 12 months (or more frequently if clinically indicated). Baseline weight is established on an individual basis in consultation with an ARC veterinarian.

2. Animals on food or fluid regulation (regardless of whether actively working or not) must be weighed at least once per week by the research team. The weight should be obtained at approximately the same time each day.

3. Each animal must be observed daily during periods of food or fluid regulation by the research team and/or animal care staff. Special emphasis should be placed on food/fluid intake, volume and consistency of stool, amount of urine (e.g., normal, no urine output) and behavior.
4. If adequately justified in an approved IACUC protocol, transient allocations of less 50% of ad lib daily calories or less than 20ml/kg/day may be considered short term “maximal restriction”. When utilizing this level of restriction, veterinary staff must be notified in advance. During these periods, the research staff will need to perform and record close monitoring of food and water intake, quantity and consistency of stool, urine output, behavior, activity level, and body condition on a daily basis. Parallel assessments will be made by ARC technologists or veterinarians throughout the duration of the maximal restriction.

   a. Physical signs that may be evidence of dehydration or inadequate caloric include:

      • drinking urine,
      • anorexia,
      • scant or no urine output,
      • scant hard feces,
      • reduced skin turgor or dry mucous membranes,
      • behavioral changes such as lethargy or incoordination.

   b. NOTE: Acute dehydration can cause poor task performance, so rather than assuming performance changes are due to insufficient motivation, these changes need to be considered within the context of the other findings.

5. If an indication of potential dehydration or inadequate caloric intake is identified by researchers, animal care or veterinary staff, a veterinarian must be notified. Based on their observations, a physical exam +/- blood and/or urine analysis will be performed as indicated. The baseline data obtained from blood samples prior to restriction and/or charts of normal values will be used as a comparator to determine whether the level of restriction is causing excessive changes in parameters such as serum osmolality, sodium concentration, urine ketone content and urine osmolality as described in the APV Guidelines. Adjustments to the water allocation to rectify dehydration or an adjustment to biscuit count to rectify evidence of a clinically significant caloric deficit will be made as necessary under the direction of an ARC veterinarian.

6. If anorexia occurs in conjunction with a high level of water restriction, this must be reported to the veterinary staff and efforts to address it must be taken, such as providing moistened biscuits and ensuring that part of the water allotment is given at the same time food is offered. Continued inappetence is an indication that the animal is not successfully adapting to the level of water control and the restriction must be lessened to allow normal food intake.

7. If an animal drops below 90% of the veterinary calculated baseline-weight while on food or fluid regulation, a veterinarian must be contacted to assess the animal’s body condition and physical well-being. If an animal’s weight remains below 85% of the baseline weight for 24 hours despite intervention, the animal must be given ad libitum access to water until its weight has increased to greater than 90% of baseline.

8. Animals must be given unrestricted access to food or fluid if there is >15% body weight loss from baseline, the BCS is <2.5/5 for adult NHPs (macaques 8 or older, marmosets 1.5 or older) or < 2.0 for subadults (macaques <8, marmosets <1.5; see Figure 1), significant abnormal behaviors have developed, or clinical chemistry parameters are significantly out of normal range. The animal may be returned to study with approval from a veterinarian when improvements in the abnormal parameters.
Section D – Record Keeping

Fluid regulations records must be maintained as follows:

1. Body weight must be logged by the research team a minimum of once each week while an animal is on food or fluid regulation (including initial training).

2. For fluid restriction, juice preferences should be documented for each animal. For each working session, the animals’ minimum fluid volume, the volume earned during work, and the volume supplemented must be individually documented daily to help assess if adjustments should be made to the task.

3. For food restriction, food preferences should be documented for each animal. For each working session, the animal’s minimum caloric intake, caloric consumption earned during work, and calories supplemented should be documented to assess if adjustments should be made to the task.

4. Records (e.g., animal medical records, feeding or fluid logs, behavior assessments, etc.) must be available for review by the IACUC, ARC veterinary staff, and post-approval monitoring staff in the animal housing room.

Section E - Protocol Considerations

The following points related to fluid regulation must be adequately addressed in the IACUC protocol:

1. Is fluid regulation essential to address the scientific objectives stated in the protocol? If so, how can fluid regulation be limited to the minimum required to meet the scientific objective?

2. Although a minimum volume of 20 ml/kg/day should be used as a restricted volume after the initial training period, the minimum restriction possible should be implemented for each individual animal. Describe how the amount of fluid provided will be determined for each animal.

3. Describe how alternatives to minimize/eliminate fluid restriction will be explored (food/ treats/ juice).

4. What is the plan to ensure the animal’s physiologic and psychological well-being? What parameters will be used to monitor the health and well-being of the animal (e.g., body weight shifts and body condition scoring, changes in serum/plasma or urine osmolality, among others)?

5. Describe how animals will transition from unrestricted to restricted water access (describe step-down process, which should be completed over several days). Describe how animals will transition from restricted to unrestricted water access in the case of vacation periods or other breaks.

6. Once an animal has learned a task, opportunities to complete the task with less fluid regulation should be incorporated. Describe how this will be done (this is often accomplished by increasing the reward provided for each successful task).

7. Will supplemental fluid be given on days when work is not scheduled (i.e. at the end of study or during prolonged periods of inactivity)? If not, describe how it was determined that this is not scientifically feasible.

8. Describe the minimum length and number of water vacations animals on restriction will receive each calendar year.
9. What is the intervention plan for animals failing to meet the established health parameters (e.g., supplemental fluid or restoration of ad lib fluid consumption)? What is the humane endpoint for fluid regulated animals?

**Body Condition Chart for Laboratory Primates**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
<th>Illustration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>EMACIATED</strong> – Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal to no muscle mass is palpable over ilium or ischium. Anus may be recessed between ischial callouses. Body is very angular, no subcutaneous fat layer to smooth out prominences.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>1.5</td>
<td><strong>VERY THIN</strong> – Hips, spinous processes, and ribs are prominent. Facial bones may be prominent. There is very little muscle present over the hips and back. Anus may be recessed between ischial callouses. Body is angular, no subcutaneous fat to smooth out prominences.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>2</td>
<td><strong>THIN</strong> – Very minimal fat reserves, prominent hip bones and spinous processes. Hips, spinous processes and ribs are easily palpable with only a small amount of muscle mass over hips and lumbar region.</td>
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<tr>
<td>2.5</td>
<td><strong>LEAN</strong> – Overlying muscle gives hips and spine a more firm feel. Hip bones and spinous processes are readily palpable, but not prominent. Body is less angular because there is a thin layer of subcutaneous fat.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
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<tr>
<td>3</td>
<td><strong>OPTIMUM</strong> – Hip bones, ribs and spinous processes are palpable with gentle pressure but generally not visible. Well developed muscle mass and subcutaneous fat layer gives spine and hips smooth but firm feel. No abdominal, axillary or inguinal fat pads.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>3.5</td>
<td><strong>SLIGHTLY OVERWEIGHT</strong> – Hip bones and spinous processes palpable with firm pressure but are not visible. Bony prominences smooth. Rib contours are smooth and only palpable with firm pressure. Small abdominal fat pad may be present.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
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<tr>
<td>4</td>
<td><strong>HEAVY</strong> – Bony contours are smooth and less well defined. Hip bones, spinous processes and ribs may be difficult to palpate due to more abundant subcutaneous fat layer. May have fat deposits starting to accumulate in the axillary, inguinal or abdominal areas.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
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<tr>
<td>4.5</td>
<td><strong>OBESE</strong> – This animal will often have prominent fat pads in the inguinal, axillary or abdominal region. Abdomen will be pendulous when animal sitting or ambulating. Hip bones and spinous processes difficult to palpate. Bony contours smooth and poorly defined.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
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<tr>
<td>5</td>
<td><strong>GROSSLY OBESE</strong> – Obvious, large fat deposits in the abdominal, inguinal and axillary regions. Abdominal palpation is very difficult due to large amount of mesenteric fat. Pronounced fat deposits may alter posture/ambulation. Hip bones, rib contours and spinous processes only palpable with deep palpation.</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
</tbody>
</table>
Section F – References


