COMPARATIVE MEDICINE
LABORATORY ANIMAL FACILITIES

STANDARD OPERATING PROCEDURES FOR FOOD AND FLUID RESTRICTION/DEPRIVATION GUIDELINES

1. Introduction:

Food and fluid restriction or deprivation may be required to conduct some physiologic, neuroscience, and behavioral research protocols. The process may entail scheduled access to food or fluid resources, so an animal consumes as much as desired at regular intervals or the total volume of food or fluid consumed is strictly monitored and controlled. Food restriction in the research setting is analogous to the normal wild life setting. There is still ongoing debate as to whether or not food or fluid restriction is best to achieve results in behavioral studies. A protocol is considered appropriate if animal discomfort is kept to a minimum while motivation for the task is maximized. The Guide for the Care & Use of Laboratory Animals (8th ed.) recommends the use of highly preferred foods or fluids as positive reinforcement, instead of restriction or deprivation.

2. Definitions:

Water restriction: The **total** volume of fluid consumed is strictly monitored or controlled. Animals are fed their full daily ad lib diet.

Water deprivation: The withholding of water for a specified period of time in a structured experimental situation. At the end of the designated period, animals are returned to free access of water.

Food restriction: The **total** amount of food consumed is strictly monitored or controlled each day for a prolonged period of time.

Food deprivation: The withholding of food for a specified period of time in a structured experimental situation. At the end of the designated period, animals resume ad-lib feeding.

3. General Procedures:

3.1 Planned duration of food/fluid deprivation/restriction must be specified, scientifically justified, and approved in the IACUC protocol.

3.2 The investigator must use the least possible restriction to achieve their specified scientific objective(s).

3.3 At the end of the testing period, the animals must be returned to free access of food/water or be euthanized.

3.4 The PI may consult with the Veterinary Staff before starting a new food/fluid restriction study. CMLAF places the animals “on report” to ensure that they are monitored regularly by the veterinary staff.

3.5 Rodents on food/fluid restriction or special diet must be identified by a green card on their cage(s) (Appendix 2). The animal caretaker must be informed of this schedule. If for any reason the animals cannot be fed/watered, the CMLAF must be informed immediately.

3.6 Animals should be singly housed to eliminate competition for limited food/water resources.

3.7 Parameters such as body weight, hydration status, body condition, and food consumption should be closely monitored and accurately documented on a chart. This chart must be kept in the animal holding room (see Appendix 3) and initialed to indicate that the animals are cared for each day. The chart must be prepared and left in a conspicuous place in the animal holding room.

3.8 Weight/growth charts or an ad lib fed control animal of same strain, age, and sex should be used as the basis to determine percentages of weight loss. Typically 85% of free-feeding weight provides adequate motivation for behavioral studies. Alternatively, weight gain - trajectory based on the initial weight of the animal can be used. Sensible targets in rats could be 2gms/week increases in males and 1gm/week in females.

3.9 Restriction/deprivation studies should take into consideration that female rats/mice have natural 4 day cycles of food intake and body weight changes.

3.10 If treats are used as supplements or reinforcers, they should be nutritious and contain protein and micronutrients. Milk or dairy products should never be used.

4. Fluid Restriction:

4.1 Animals should be acclimated gradually, over a period of at least 3 days, to a new restriction regime.

4.2 Most animals adapt well to once-daily water access.

4.3 Animals should be allowed sufficient time each day to consume some food while water is available to maintain weight. (An acceptable time is 20-60 minutes.)

4.4 Complete deprivation of fluids for more than 24 hours should be avoided as much as possible. Greater than 24 hours dehydration may lead to substantial anorexia for some species, or death in mice. However, rats have been known to tolerate restrictions of up to 72 hours.

4.5 Rodents will reduce food consumption during water deprivation referred to as “dehydration anorexia” to conserve water loss and reduce intake of electrolytes. Therefore loss of BW is a reflection of decreased food in the GI tract and may not be an accurate indicator of dehydration.

4.6 It is important to establish mean daily water intake for the particular strain/species to be used in each study. (Compare to a control animal on ad lib feeding/watering or charts supplied by the vendor.)

4.7 A low fiber diet (e.g., 2019 Teklad diet for rodents), should be considered. Commercial diets normally have a high fiber-content which contributes to an increased loss of water in their fecal matter.

4.8 Young or growing animals (rodents less than 2 months old, rabbits and larger species less than 6 months old) are especially sensitive to fluid restriction. These classes of animals, placed on fluid regulation/restriction, must be evaluated with due concern for their health and minimum growth requirements.

4.9 Genetically altered mice with impairments in fluid conservation or balance may need more careful consideration.

4.10 Because most food intake occurs at night, overnight water deprivation with food produces the same full effects of dehydration as 24 hours of water deprivation. It is suggested that water be offered during the lights-off cycle.

5. Food Restriction:

5.1 Food restriction may be based on weight of a complete diet or by energy content of a diet.

5.2 Most animals adapt well to food being offered once daily, but should be provided adequate time each day to eat food while water is available. (An acceptable time is 20-60 minutes)

5.3 Animals subjected to dietary restriction should be appropriately monitored for weight loss. The investigator is responsible for assuring that specially formulated diets are nutritionally adequate and palatable. Nutritional information should be made available to the IACUC/CMLAF veterinarians prior to initiating a protocol or protocol amendment.

5.4 Water must be available during food consumption. Animals fed without accessible water or fed and watered out of schedule will eat less and lose weight.

5.5 Use of reverse light-dark cycles may be used for restriction since rodents are more active at night and tend to consume most of their food during the dark phase of the day. This will allow investigators to monitor food consumption more closely. Although rodents can adjust, food removed during the light phase is not as effective for restriction (since they normally are sleeping during this phase), as removing food in the dark phase of the cycle.

5.6 Larger and heavier/fatter animals (rats) are better able to cope with food restriction than smaller animals (mice).

5.7 For food deprivation and restriction studies, higher ambient temperature will result in metabolic energy savings and less weight loss, therefore, warmer room temperatures should be considered.

6. Pre-anesthetic Fasting:

6.1 For many species, food may be withheld for a specified period of time prior to surgery in order to prevent vomiting and aspiration of food while sedated or under anesthesia.

6.2 For large animals, food may be withheld overnight prior to an anesthetic procedure (i.e., overnight fasting). Water should be available during the overnight fast but may be removed in the morning on the day of surgery.

6.3 In most cases, pre-anesthetic fasting is not required for rodents, birds, and rabbits. However, specific surgical procedures may require an overnight fasting to empty the stomach and should be explained in the IACUC Protocol.

7. Conclusion

7.1 If the PI staff is feeding or watering the animals, a chart must be left in the animal holding room and indicate that this has been performed each day including weekends and holidays.

7.2 Endpoints should be specified in advance on the approved protocol. Examples of specific endpoints are:

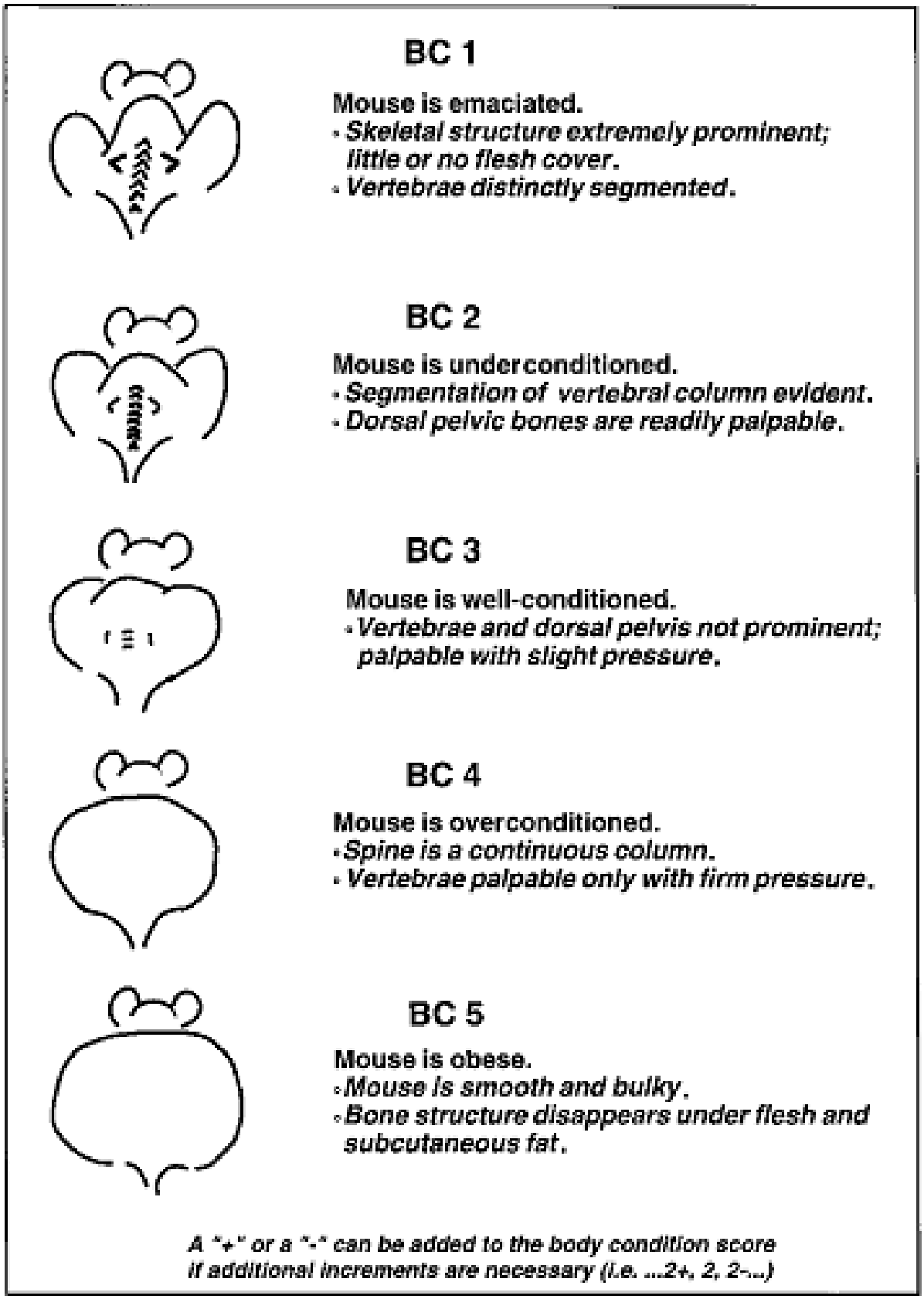
7.2.1 Failure of growing animals to gain weight based on weight/growth charts.

7.2.2 Loss of greater than 20% of the body weight compared to a conspecific.

7.2.3 A body condition score of 2 or less indicating an animal is emaciated (segmentation of the vertebral column and dorsal pelvic bones are readily palpable; see Appendix 1).

Appendix 1:

The body condition can be scored on a scale of 1 through 5:



Appendix 2

Cage Card

DO NOT FEED

ANIMAL ID/ CARD #_____

BEGIN FAST: _____

DATE TIME

RETURN FEED: _____

DATE TIME

SPECIAL DIET/WATER

PLEASE FEED ☐ PLEASE WATER ☐

CAGE CARD NUMBER(S) _____

Time Period

From _____ To_____

TYPE OF DIET/WATER _____

Requested By: _____

Appendix 3:

University at Buffalo
The State University of New York

Division of Comparative Medicine and Laboratory Animal Facilities

Feeding and Watering Recording Log
For
Investigators Feeding or Watering Their Animals

Date	Animals Fed	Animals Watered	Hydration Status	Body Weight	Body Condition Score	Food Consumption	Initials	Comments

Investigator _____ Protocol # _____Room #_____