IACUC Standard Operating Procedure SOP-01-2015: Food Restriction in Rodents

**Rats:** Subjects will be restricted to a limit of 85% of their age-based free feeding body weight, using age/sex-weight charts from the vendor or age/sex-matched controls* on *ad lib* food. Subjects will be weighed at the outset of restriction, and then at least 3 times/week thereafter during restriction. Record sheets should reflect an initial weight (at the start of testing), and the source of age-match weight data. In addition, the record should note for each weighing: 1) date, 2) actual weight, 3) age-adjusted base weight, and 4) % for actual weight/base weight. If these records are kept in a testing room during active research, a note will be left in the Housing room as to where the notebook can be found. At the conclusion of testing daily, the weight notebook will be returned to the housing room. Cages of food-restricted animals will be demarcated in some way (e.g., by colored cards). Animals on food restriction are singly housed to better control food intake of individual animals.

To achieve a reduction to 85% baseline weight, rats will receive 5g/day per 100g body weight (roughly 80-85% normal intake), with an expectation that target weight (85% ad lib) is reached in approximately 7 days (plus or minus a few days). Food can be allocated using the known mean weight of an individual pellet, and split pellets can be used as needed. Where calculated increment amounts <1/4 pellet occur, the assigned pellet portion will be rounded up.

For short breaks in the experiment (<4 days), the animal will be given extra food (about 5gr) on the day preceding a break day. Extensive breaks in testing (4 days +) will allow return of *ad lib* food, until re-initiation of restriction to achieve target weight before testing resumes.

Restriction will last for the minimum duration needed to achieve the scientific goals which is: Periodically in addition to daily visual inspection during testing (coat quality, eye/nose secretions, demeanor/posture), animals will be manually palpated for body score if possible, and these notes will be included in weight records. Any animals that exceed the allowable % weight loss or show other signs of significant distress (porphyrin stained eyes/nose, ruffled coat, lethargy) will be removed from restriction (moved to *ad lib* feeding) and referred to the ACS veterinary staff for evaluation.

When referenced in protocol, a copy of this SOP should be posted in the testing room where food restricted animals are tested.
**Mice:** Subjects will be restricted to a limit of 85% of their age-corrected body weight using age/sex-weight charts from the vendor or age/sex-matched controls* on *ad lib* food. To achieve the 85% of base weight, mice will be restricted to 70% normal intake. In a normal adult 25-30g mouse this represents 2-4g (70% of 3-6g normal intake). Food will be allocated based on the average weight of a pellet, and use of split pellets. Where smaller increment calculated amounts occur, the assigned pellet portion will be rounded up.

Record sheets should reflect an initial weight (at the start of testing), and the source of age/sex-match weight data. In addition, the record should note for each weighing: 1) date, 2) actual weight, 3) age-adjusted base weight, and 4) % for actual weight/base weight. If these records are kept in a testing room during active research, a note will be left in the Housing room as to where the notebook can be found. At the conclusion of testing daily, the weight notebook will be returned to the housing room. Cages of food-restricted animals will be demarcated in some way (e.g., by colored cards). Animals on food restriction are singly housed to better control food intake of individual animals.

For short breaks in the experiment (<4 days), the animal will be given extra food (about 2-3gr) on the day preceding a break day. Extensive breaks in testing (4 days +) will allow return of *ad lib* food, until re-initiation of restriction as needed to achieve target weight before testing resumes. Restriction will last for the minimum duration needed to achieve the scientific goals, which is:

Periodically in addition to daily *visual* inspection during testing (coat quality, eye/nose secretions, demeanor/posture), animals will be manually palpated for body score if possible (noting that in anxious/squirmy mice this may not be possible). Notes should be kept in the weight notebook. Any animals that exceed the allowable % weight loss or show other signs of significant distress (/nose, ruffled coat, lethargy) will be removed from restriction (moved to *ad lib* feeding) and referred to the ACS veterinary staff for evaluation.

When referenced in protocol, a copy of this SOP should be posted in the testing room where food restricted animals are tested.

*Use of *ad lib* fed age/sex-matched controls in food restriction procedures, rats and mice.*

The allowance for use of age/sex-matched *ad lib* controls in lieu of vendor-provided weight data is intended to address 2 scenarios: (1) No weight data is available. This may be the case when extended ages are being used, or a new preparation for which vendor weight data has not been gathered. (2) When an additional variable is employed that might be expected to impact baseline weights for all animals in the study, outside of food restriction procedures. Examples might be animals used in exercise studies, or on special diets simultaneous to behavioral assessment requiring food restriction. In these cases, use of *ad lib* age/sex matched controls can provide data on baseline weights in the presence of the background variable.

When age/sex-matched *ad lib* controls are used to determine baseline weights for food restriction, it is required that sufficient data is gathered to generate a weight curve that meets 90% reliability. Typically, this would require weight measures from at least 4 independent *ad lib* animals of each sex, taken at a minimum of every 7 days over the period of food restriction. If
the vendor provides restricted weight data (e.g., <100 days only), and the PI requires a curve extending to older ages, it is expected that the PI append a best-fit curve extending *from* the vendor curve, while leaving the vendor curve *intact*. (The highest age available should be used as a fixed start point for the extended curve). From these data, a best-fit curve can be derived for use at interpolated ages. A statistical validation of the new weight data, with reliability indices, must be included in the protocol. If age/sex-matched controls are bought at the time of study, and no best fit curve can be generated *a priori*, then the *ad lib* controls must be weighed and averaged *every day* that weight-restricted animals are weighed, with restriction targets calculated accordingly.