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*Overviews*

# Food or Fluid Restriction in Common Laboratory Animals: Balancing Welfare Considerations with Scientific Inquiry

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**Deprivation or restricted access to either food or fluids is a common research procedure in laboratory animals. The purpose of the present review is to present and summarize some of the important physiologic effects of such procedures and to assess their effect on the well-being of the animal. This assessment is presented within a context of the typical research objectives of such procedures. Specific suggestions are made that are intended to strike a balance between meeting these research objectives and ensuring the physiologic and behavioral welfare of the animals under study. Most of the information presented is specifically related to rats and mice but, with appropriate adjustments, the principles likely will generalize to other laboratory species. I present evidence that after 12 to 24 h without access, animals efficiently reduce further fluid or energy losses by a combination of behavioral and physiologic adjustments. These adjustments likely minimize the additional physiologic or psychologic stress of deprivation. Animals have endogenous nycthemeral rhythms that make them particularly adaptable to once-daily occurrences, such as food or water access. Longer periods of acute deprivation or chronic restriction are acceptable procedures, but only with suitable monitoring protocols, such as routine weighing and target weights. 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.**

**Abbreviations:** ACUC, animal care and use committee; BMI, body mass index; ECF, extracellular fluid; ICF, intracellular fluid

Animal care and use committees (ACUCs), often working with institutional veterinarians, are charged with ensuring the justified use and humane treatment of experimental animals. Some commonly encountered standards of care and treatment are uncontroversial and are relatively easy both to monitor and implement. In contrast, many experimental procedures are highly specialized and often are not within the realm of experience or expertise of either the ACUC members or the veterinary staff. The experience and judgment of the investigator are factors that might be considered, but ACUCs are far from uniform in using this information as a basis for their deliberations. To avert potential conflict with investigators, many ACUCs are developing local guidelines for a wider array of procedures. Food and fluid restriction are procedures that are essential to the conduct of certain types of physiologic and behavioral research.

The purpose of this article is first to present a perspective on what constitutes 'normal' food and fluid intake, then to discuss the physiologic and behavioral effects of restriction, and finally to present some recommendations for standard practice. This treatment will be heavily biased toward rodents because these are the most common species used in research. Most of the principles likely will generalize to other species with appropriate modification including, for example, adjustments based on relative body size and differences in natural habitat and diet.

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## Normal Feeding and Drinking

**Ecologic considerations.** The topic of this paper is restriction or deprivation, terms that can be defined only relative to what is considered 'normal.' Often, this state is implicitly assumed to be unrestricted or ad libitum access to food or fluid but, as will be discussed later, in most cases unrestricted availability is not optimal for long-term health.[26] Continual access certainly is not how animals would encounter food or water in the real world. The physiologies underlying need differ greatly for eating and drinking, so in most instances in this review, they will be treated separately.

In the case of eating, most animals have evolved in environments in which availability of food was uncertain and often insufficient. The body fat content of otherwise healthy animals killed in the wild vary from 1% to 25%, depending on species and time of year, and this range is considerably lower than that found in the same species in laboratory animal facilities.[45] Ad libitum feeding regimens of nutritious food, standard in most rodent facilities, does in fact lead to excessive fat deposition and obesity.[26,27] Ad libitum food is probably provided mainly as a convenience for husbandry staff in facilities with large numbers of rodents. In contrast, many species, including pets, zoo specimens, and livestock, often are fed only at discrete times. In those circumstances, this form of restriction is considered good husbandry. Therefore, the standard is not consistent: ad libitum food is considered normal or appropriate for some species but not for others. Although the convenience of providing food ad libitum for large colonies is

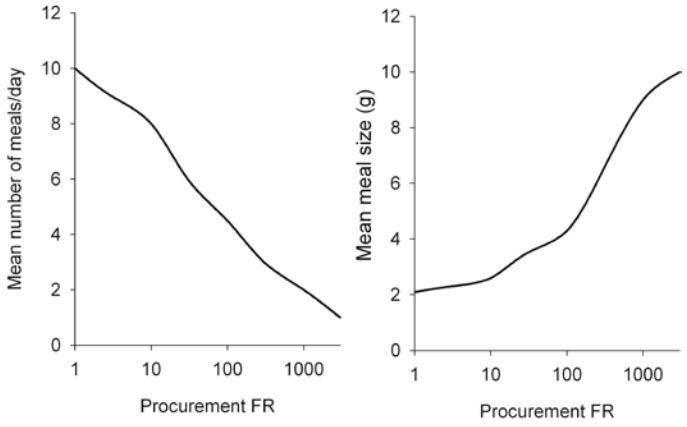


**Figure 1.** Elective change in mean number and size of daily meal in rats with ad libitum access to food but with different imposed procurement costs (fixed-ratio [FR] lever presses) for each meal. Figure is redrawn from data in reference 11.

indisputable, the use of this as a 'gold standard' against which to measure food restriction or deprivation is questionable.

**Meal or bout patterns of ad libitum food or water access.** Rodents are sometimes referred to as 'grazers,' meaning that they normally eat frequent but small meals. This term describes the behavior of rodents under conditions of ad libitum and free access to a diet of relatively high nutritional content, and is not a general or intrinsic attribute of rodents, or indeed many other species. The actual situation is that most species have flexible eating patterns, adopting different feeding strategies in different environments.

Under typical laboratory conditions of free access to a nutritionally balanced food such as a commercial chow, which yields 3 to 3.5 kcal metabolizable energy per g,[46] rats adopt a grazing pattern in which they eat about 10 meals daily. Most laboratory rodents are nocturnal, so most of these meals normally occur at night (see also the following section, *Endogenous rhythms*). The average meal size is 2 to 3 g, and so the total daily intake is 20 to 30 g (approximately 60 to 90 kcal). Figure 1 shows that rats can easily be changed from this pattern to a 'gorging' strategy. In the experiment shown,[11] rats lived in an environment in which they could work or forage for food (complete rodent diet) at any time they desired. The independent variable was 'procurement cost': the rats were required to press a lever a fixed number of times to open a door that gave them access to a food dish. They could eat as much as they wished from the dish for no additional cost but, once they left the feeder for 10 min, the door closed. In order to eat again, they would have to 'pay' another consumatory cost. Rats were studied for several days at each of a range of costs. As procurement cost increased, the mean number of meals per day decreased (Figure 1, left panel) and their size increased (right panel), so that daily total intake was approximately constant. These are all ad libitum feeding patterns because food was accessible at any and all times. The grazers became gorgers because of a small access cost, probably a modest 10 min or less of lever pressing daily. If we establish an access cost that causes an animal to eat voluntarily only 1 large meal per 24 h, it is difficult to argue that this is different physiologically from a feeding schedule in which an investigator chooses to feed an animal once daily.

Similar data have been presented for procurement costs and water intake.[33] That is, although under ad libitum conditions rats drink 20 or more bouts per day, most of which are associated in time with their grazing meals (when food has no cost), an environmental procurement cost on water can turn them into once- or twice-daily drinkers.

Returning to food, other factors such as type of diet (for example, liquid versus solid), palatability, and ease of access can affect meal patterns in rats.[11,68] Mice seem to be even more sensitive in this regard (Table 1). The number of daily meals ranges from 2 to 50, depending on the type of diet and the ease or configuration of its procurement. Even when food is available at all times, mice, like rats, choose to be grazers or gorgers as a function of what to us may seem like modest differences in the environment. Meal pattern depends mainly on factors extrinsic to the animal and affects the detection of experimental deprivation by the animal. A rodent in a grazing mode may physiologically detect the absence of food after only a few hours, whereas a rodent in a gorging mode and eating only 2 meals per day may not detect absence of food for 12 h or more.

Hamsters differ from rats or mice in that they have extremely limited flexibility in their meal size and do not compensate for infrequent availability of food by increasing meal size.[55] Instead, hamsters are particularly prodigious hoarders and in natural burrows may eat frequently from hoards.[4] Therefore, the environmental determinants of meal patterns discussed previously are modulated by species-typical constraints.

**Endogenous rhythms.** Animals have a number of endogenous rhythms ranging in duration from less than 1 d to a year. The most ubiquitous and relevant of these for animal care are 'nycthemeral rhythms,' also known as day-night or diurnal rhythms. These rhythms are temporal organizing mechanisms for physiology and behavior in all species. Most rodents are nocturnally active, so the majority of their food foraging and intake occurs at this time. For example, in the reports of mouse meal patterns summarized in Table 1, most reported that approximately 75% of the meals were taken during the lights-off phase of a 12:12-h cycle. In rats, 6 to 9 h may elapse between the last meal taken near the time of light onset and the next meal; conversely, an intermeal interval of more than 2 h is unusual during the lights-off phase.[30]

Nycthemeral rhythms are maintained by internal oscillators whose periodicity is approximately 24 h, and they normally are entrained to the light-dark cycle in a species-typical manner.[35] However, animals can readily change the timing or phase of their feeding to other times of the day-night cycle, for example if food is available only during the daylight hours or at a fixed time each day. The phenomenon of jet lag experienced by many travelers is due to the fact that the internal clock normally can only be adjusted by 10% (2 to 3 h) each 24-h cycle.[35] Likewise, nocturnal rodents require several days to adapt to either restriction of food or feeding during the daytime; such adaptation may be impaired in conditions of brain damage or drug action.[54] During restriction paradigms, animals are usually fed during the daylight hours for experimenter convenience. Using reversed light-dark cycles for feeding studies matches the time of food availability to the natural rhythm of feeding, although not all facilities may be able to accommodate this provision.

Physiologic cycles of metabolism underlie the spontaneous day-night feeding patterns of rats.[30] At night, rats eat more food than the energy they expend, and so the excess is stored as glycogen and fat (lipogenesis). By day, rats eat less food than the energy they expend (mostly basal metabolism, because they are resting), and so they mobilize glycogen and fat (lipolysis) reserves

**Table 1.** Ad libitum meal patterns in mice

| Mouse strain | Feeding configuration | Diet | No. of meals/24 h (end criterion) | Reference |
|---|---|---|---|---|
| Small (S) and large (L), inbred | Overhead door panel | Powdered rodent chow | 12 (5 min) | 44 |
| SWR/J | Recess at floor level | Powdered rodent chow | 36 (5 min) | 18 |
| C57BL/6J (lean and *ob/ob*) | Sipper spout in cage | Liquid diet EC116 | 50 (♂) 30 (♀) (various) | 59 |
| C57BL/6J (lean and *ob/ob*) | Lever press and food receptacle | Noyes 20-mg pellets | 2–10, function of access cost (10 min) | 65 |
| 129/B6 (wild type for $BNDF^{+/-}$) | Pellet removal from trough | BioServ 20-mg pellets | ~12 (18 h food access) | 16 |
| 129/B6 (wild type for $BNDF^{+/-}$) | Liquid diet from 0.02-ml dipper | Isocal high-fat liquid | ~15 (18 h access) | 16 |
| 129/B6 (wild type for $MC4R^{-/-}$) | Lever press and food receptacle (procurement cost) | Noyes 20 mg pellets | 2–7, function of procurement cost (10 min) | 63 |
| 129/B6 (wild type for $MC4R^{-/-}$) | Lever press and food receptacle (progressive ratio) | Noyes 20 mg pellets | 25–50, function of reset time (20 versus 3 min) | 64 |

via sympathetic neural and hormonal mechanisms.[30] As Friedman and Stricker[17] so vividly state "the animal with no food to consume must consume itself." These cycles of consuming from the outside and consuming from the inside are in fact natural cycles (24 h and shorter), the periodicity and amplitude of which depend on environmental factors (for example, as in grazing versus gorging).

Like food intake, spontaneous or ad libitum water intake in rats is mainly nocturnal, most of it associated with meals.[28] Spontaneous water intake can to some extent be dissociated from availability of food: when food intake of rats was equally rationed between day and night, water intake was still 75% nocturnal.[37] However, the timing of food intake when water is restricted may be less determined by the day-night cycle, in part because dehydration causes anorexia (see the section titled *Water deprivation and body fluids*).

Some laboratory animals (for example, hamsters, squirrels, some avian species) show circannual rhythms, even in a laboratory with constant temperature and day-night cycle. One feature of these species is that they gain weight at 1 phase of the year and lose weight at another, and studies using food restriction may need to take this natural variation into account.

Another cycle is that related to the hypothalamo-pituitary-gonadal axis in female animals. In rats and mice, estrous cycles normally are 4 d in duration, characterized by increased plasma concentrations of estradiol and progesterone, increased locomotor activity, and decreased food and water intake. Most of these effects are due directly to estradiol.[13] Therefore, female mice and rats will have natural cycles of 4-d food intake and body weight, and studies using food deprivation or restriction may need to account for this variation by prior monitoring to determine or control the phase of the cycle at which experiments will be conducted.

**Set points.** The mean values around which body weight oscillations occur often have been called 'set points' by analogy with the engineering systems that are designed to regulate variables such as temperature via thermostatic control system in an animal facility. A person sets the desired temperature, and the system is designed to deliver an acceptable oscillation around that set point. This engineering concept translates reasonably well to biologic systems that have little or no capacity for storage; such systems include respiration, fluid homeostasis, and thermoregulation.

Feeding or energy balance is a quite different matter, because of the enormous capacity for storage and mobilization, as mentioned previously. For a given level of food or energy intake, energy expenditure will stabilize at a particular level, and this level will be associated with a particular size of the energy stores. Figure 2 is a theoretical analysis of a situation that does not include a set point for stores or body weight[22] and shows that in principle an infinite number of steady states are possible. Therefore the concept of set or settling point for body weight[20,38,69] has little or no meaning without specifying the environment to which it refers. Relevant environmental factors include but are not limited to the nutritive value or composition of food, its palatability, the relative effort expended in food procurement, ambient temperature, and availability of water.

A demonstration of the flexibility of body weight is the paradigm of dietary obesity in which rodents fed a highly palatable, calorically dense diet eat more calories and gain body weight and fat compared with chow-fed controls.[62] Conversely, poor tasting or calorically dilute diets may be consumed in smaller amounts and produce lower body weights.[43]

Rodents and other species that are maintained on a monotonous or bland diet such as chow will avidly consume a palatable treat that is presented for a short time each day. Such treats can be used as experimental meals[57] or as environmental enrichment.[51] In the latter study, mice compensated for the calories in the treat by reducing their ad libitum intake of chow by the same number of calories. However, if the treat provides high caloric yield, either because of its high fat content or an extended period of availability, then fully compensatory caloric reduction in chow intake may not occur. Treats presented either as meals or as enrichment generally are not intended to produce dietary obesity.

## Deprivation and Restriction

Toth and Gardiner[61] drew an important distinction between deprivation and restriction, and I will discuss that general distinction as well.

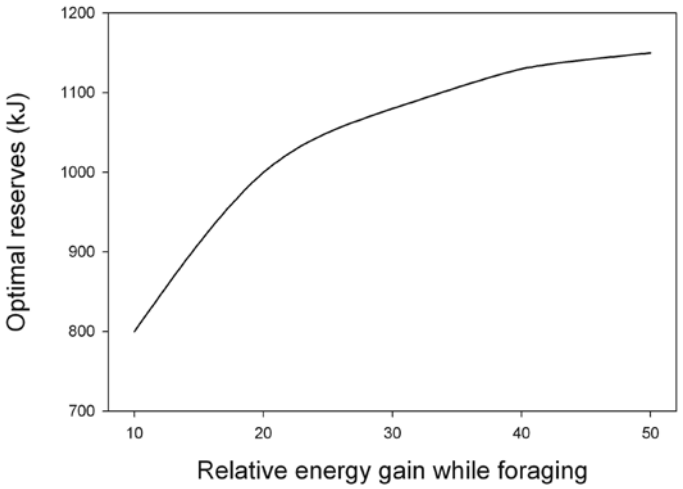


**Figure 2.** Optimal food reserves (mostly as triacylglycerols in adipose tissue) as a function of relative energy gain from foraging in a theoretical model of energy flow into and out of a virtual animal comparable in size and physiology to a rat. The relative energy gain is related to the energy in the food minus the energy expended to obtain that food. Note that expected stable body weight is a continuous function of net energy gain; no body weight ideal or set point is built into the model. Figure is redrawn from data in reference 23.

**Deprivation studies.** Many protocols are designed to evaluate the behavioral or physiologic effect of withholding a commodity for various periods of time. The overwhelming majority of studies in the literature has used multiples or submultiples of 1 d (for example, 6, 12, 24, 48 h). These are known as 'deprivation studies' because at the end of the designated period, the animals are tested and returned to free access of the commodity or, in some cases, are euthanized. Animals may experience some discomfort during longer deprivations, but the deprivation period has a defined end time. For this type of study, the issue before the ACUC is the scientific justification for a particular duration of deprivation within the context of potential distress or physiologic harm.

**Restriction studies.** Restriction studies, in contrast, do not involve complete withholding of a commodity but rather the presentation of a controlled ration each day for a more or less prolonged period of access. Most studies of this type use the restriction protocol to reproduce a consistent state of physiologic need from day to day such that behavior will be motivated and stable. This state is a prerequisite for scientific evaluation of hedonics, reinforcement, and other higher cognitive and motivational characteristics in animals. The chronic nature of these studies could have adverse effects over time; the health status of such animals should be monitored, for example by weighing or physical exam, and observations or measurements recorded at intervals deemed appropriate by the ACUC. Restriction studies normally are performed using healthy animals in which the physiologic consequences differ from those of anorexia due to illness. A healthy animal that has lost 15% body weight by restriction is likely to be in a clinically stable condition, whereas one that has lost the same weight due to illness is not.

**Environmental enrichment considerations.** The *Guide for the Care and Use of Laboratory Animals*[40] recommends that social housing is the best form of environmental enrichment for social species, including rodents. Social or other enrichment can affect the impact of restriction or deprivation in several ways. For example, social housing is not recommended for animals on restriction because of the risk of fighting and/or because unequal competition for a ration offered to a group of animals will introduce uncontrolled variance in the intake of the individuals in that group.

**Table 2.** Body fluid compartments and constituents in humans

| Physiologic property | Intracellular | Extracellular[a] |
|---|---|---|
| Volume (% body weight) | ~40% | ~20% |
| $Na^+$ (mEq/l) | 12 | 145 |
| $K^+$ (mEq) | 150 | 4 |
| $Ca^{++}$ (mEq/l) | <0.001 | 5 |
| $Cl^-$ (mEq/l) | 5 | 105 |
| Phosphates ($P_i$, meq/l) | 100 | 2 |

[a]The extracellular fluid compartment is ~75% interstitial fluid and ~25% blood plasma. Values for most mammals, and in particular rats and mice, are quite similar.

The use of a modest amount of a palatable and nutritious food treat[51] may be incorporated into some restriction studies. The use of chewable objects may also be acceptable, although whether their provision affects food motivation has not been assessed formally. The use of larger cages or activity devices may affect energy expenditure during food deprivation, but this possibility also has not been evaluated comprehensively. In-cage shelters such as polyvinyl chloride pipes or other plastic enclosures are well used by rodents,[19] but whether this use affects energy loss during food restriction, for example by reducing local heat loss to the room, has not been examined. My laboratory[51] examined weight gain in mice with or without plastic 'igloo' shelters and on 2 diets and found no significant effects of the shelters. However, the generality of that finding must be established in a wider range of conditions before it can be recommended for routine or general use.

## Physiology of Water Deprivation and Restriction

**Body fluid compartments.** Body fluids are distributed both inside of cells (intracellular fluid, ICF) and outside of cells (extracellular fluid, ECF). The relative sizes and ionic compositions of these 2 compartments are shown in Table 2. Fluids are lost continuously in urine and feces, as sweat, and by respiration.[7,15,56]

Water and solutes move between ICF and ECF under the force of osmotic pressure. Net movement does not occur when the osmotic pressure is equal on both sides of cell membranes. Osmotic pressure is normally about 290 mOsm/l, which is often known as 'isotonic.' ECF has both interstitial and plasma components. These are similar in composition because they are connected through the relatively porous junctions between endothelial cells that comprise capillary walls, but molecules such as plasma proteins (for example, albumin) are trapped in the vasculature because they are too large to leak out. The concentration of plasma protein rises if ECF is lost from the vasculature, thus providing a useful indicator of intravascular dehydration.

ECF is filtered into tubules in the kidney; the amount of urine formed at the other end of the nephron is critically dependent on 2 main types of reabsorptive processes that occur in the tubule. First, sodium (along with chloride and water) is reabsorbed in the proximal tubule; this process is stimulated by aldosterone. Second, water is recovered in the distal tubule by the action of vasopressin (antidiuretic hormone) at V2 receptors, thus concentrating the solutes in the remaining tubular fluid, which becomes urine. The maximal concentration of urine that can be achieved by rodents is approximately 3000 mOsm/kg water, about 10×

**Table 3.** Effects of various durations of water deprivation on activity, food intake, body weight loss and recovery in Wistar rats

| Duration of water deprivation (h) | Home cage activity (%) | Food intake (% baseline) | Weight loss (% baseline) | Days to weight recovery (99%) |
|---|---|---|---|---|
| 0 (baseline days) | 100 | 100 | 0 | 0 |
| 24 | 96 | 88 | 4 | 1 |
| 48 | 104 | 28 | 8 | 3 |
| 72 | 99 | 14 | 11 | 5 |
| 96 | 65 | 14 | 13 | 9 |

Values are derived from graphical data presented in reference 1. Home cage activity and food intake are changes across time in a single group deprived for 96 h. Weight loss and recovery data (to 99% of growth-extrapolated baseline of ad libitum group) are from 4 separate groups differing in duration of deprivation.

isotonic, but may decline with age.[6] The physiologic impact of water deprivation will depend critically upon the magnitude or efficacy of these 2 mechanisms of fluid conservation. These processes are extremely efficient in many species, although genetic or other experimental manipulations have the potential to reduce this efficiency, and such animals should be monitored particularly carefully.

**Water deprivation and body fluids.** The physiologic effects of water deprivation are critically dependent on composition of the maintenance diet and whether it is available during the deprivation period. Most rodents are maintained on a commercial chow formula. Purina Mills Inc. reports that their #5001 diet contains (by weight) 0.40% $Na^+$, 0.95% $Ca^{++}$, 1.10% $K^+$, 0.65% $Cl^-$, and 0.67% P.[46] Diets like this have a fairly high fiber content and produce high fecal volume and corresponding fecal water loss (approximately 2 g/g fecal solid), amounting to approximately 10 ml/d in an adult rat, or 25% of their total daily water loss.[47] With a low-fiber synthetic diet, fecal volume and water loss is much lower.

In addition to the hormonal conservation responses mentioned in the section on body fluid compartments, a critically important behavioral response that rodents make during water deprivation is to reduce food intake. This compensatory action is referred to as 'dehydration anorexia.'[66] This action serves 2 purposes: first, it reduces the quantity of electrolytes that enter the body and ultimately would have to be excreted, and second, it reduces obligatory fecal volume and the associated water loss. This anorexia becomes progressively greater with increasing duration of deprivation. For example, Bealer and colleagues[5] reported that chow intake of male rats fell to approximately 65% and 30% of the ad libitum level during the first and second 24 h of water deprivation, respectively. Comparable data were reported by Armstrong and colleagues,[1] who extended the deprivation to 96 h (Table 3). They found that food intake was even lower on the 3rd and 4th days of water deprivation but that body weight loss slowed from 4% on each of the first 2 days to 3% and 2% on days 3 and 4.[1] Loss of body weight therefore is not an accurate index of water loss or dehydration, because most of the loss is the result of anorexia and decreased food volume in the gastrointestinal tract. Weight loss may be more useful as an index of nutritional status. In rats, the weight loss observed after 96 h of water deprivation (13%) is approximately the same as that after 48 h of food deprivation,[1] suggesting that water deprivation is physiologically less stressful than food deprivation. Activity level in that study did not decrease until the 4th day of water deprivation (Table 3), further suggesting that these animals remained physically capable for at least several days.

The urinary exchanges reported by Bealer and colleagues[5] during 48-h water deprivation in rats with chow available are shown in Figure 3. Urinary volume was reduced by more than 50% during the first 24 h, yet urinary concentration (osmolality) was only slightly increased. This difference is due to the large reduction in food intake and to a urinary output of $Na^+$ and $K^+$ that exceeded the intake in food. Others have reported that the natriuretic effect of water deprivation, amounting to 3 to 4 mmol/kg after 24 h in rats, is similar in rabbits, sheep, and dogs,[32,34] and that it occurs using a variety of diets.[33] This pattern is a direct effect of dehydration and not the associated anorexia because comparable natriuresis was not observed when hydrated rats were pair-fed to a water-deprived group.[32] When rats were water-deprived without food available, the physiologic changes were qualitatively similar to but smaller than those in rats deprived with food available.[5]

This urinary 'solute dumping' along with reduced food intake and fluid loss minimize the effect of water deprivation on body fluid homeostasis. During water deprivation in rats with food present, the increases in plasma osmolality were only a modest approximately 2% and 4% after 24 and 48 h, respectively;[5,53] the corresponding decreases in plasma volume were approximately 4% and 13%, respectively. Changes in parameters of hydration after 24-h water deprivation in dogs, monkeys, rabbits, sheep[32,53] are similar to the cited rat data and may be less than in humans.[61]

Mice have considerably smaller body size and correspondingly faster water turnover, compared with rats. In a survey of 28 strains of mice fed chow,[2] the mean daily water intake varied by 2-fold across strains but averaged 7.7 ml/30 g body weight, or about 25% of their body weight. In contrast, rats drink approximately 10% of their body weight daily.[28] Mice also show larger changes with dehydration. Therefore, 24-h water deprivation of various wild-type or control strains of mice with dry food available produces weight losses of 6.7% to 9.5%[21,50,52] and a 6.2% increase in plasma osmolality.[52] Both of these changes are about 2-fold greater than those summarized previously for 24-h water-deprived rats.[5] The relative anorexia during water deprivation in mice[50] was comparable to that reported for rats. Mice with a genetically engineered change in a component of fluid balance may show impairments in fluid conservation or other aspect of fluid homeostasis, and so studies that include such strains should consider carefully the additional physiologic effect of a given duration of water deprivation.[36] Because 24 h of water deprivation has apparently larger physiologic effects in mice than rats, investigators planning to extrapolate from rat to mouse studies should be aware of this difference. In the absence of comparative time-course data on the physiologic effects of water deprivation in mice versus rats, weight loss may be a suitable indirect criterion or target.

A detailed time-course of changes during deprivation has been reported for dogs, in which repeated blood sampling is feasible without undue impact on hydromineral balance. Reviewing these

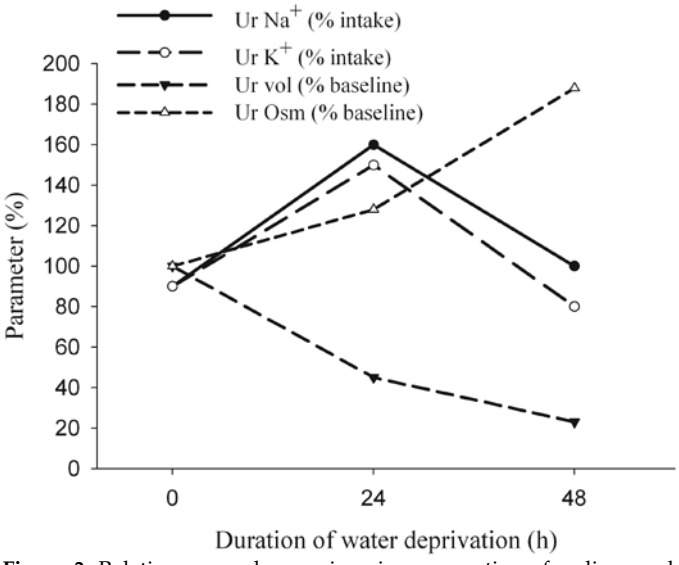


**Figure 3.** Relative mean changes in urinary excretion of sodium and potassium (both as % of the intake of these cations in food available during this period) and volume and osmolality of urine (as % baseline) in rats during 2 consecutive 24-h periods of water deprivation. Figure is redrawn from data in reference 5.

data may help inform rodent studies. Parts of these data[34] are redrawn in Figure 4, which shows changes in blood indices of fluid balance across 24 h of dehydration and then during 24 h of rehydration. Not shown are values from a preceding baseline day which, for the most part, were steady at or near the initial values for the dehydration day. Figure 4 shows that during water deprivation, plasma osmolality increased rapidly and peaked at 7% to 8% above baseline after approximately 10 h; no further increase occurred after that time. Plasma sodium concentration showed a similar trajectory (data not shown), and plasma vasopressin levels followed osmolality quite closely. This association is expected because vasopressin secretion is under the direct control of osmoreceptors.[15,56] In contrast, the concentration (or, more accurately, the enzymatic activity) of plasma renin, a hormone secreted in response to decreased plasma volume, increased more slowly during deprivation. Aldosterone, a hormone of sodium deficiency, was unchanged. Therefore, plasma osmolality appears to be the earliest indicator of dehydration. However, the relative timing and magnitude of these changes may have been affected by feeding. The dogs received a moist ration at time 0 on all days and consumed it within a few minutes. If the diet had been provided at a later time of day, then the rise in these parameters presumably would have been correspondingly later.

On the rehydration day, the dogs consumed approximately 1500 ml (and their food ration) during the first 2 h, and the excess water ingested balanced their deficit from the deprivation period. As a result, plasma osmolality and vasopressin returned rapidly to normal (Figure 4). In contrast, renin remained high for more than 12 h, and aldosterone showed a large and enduring increase, presumably in part to retain sodium lost during deprivation, and with the eventual effect of restoring plasma volume.

Acute depletions of either the ICF or ECF evoke thirst. These effects can be studied independently, and such experiments show that the threshold for ICF or cellular dehydration is approximately 2% whereas that for ECF or extracellular dehydration is typically a little higher.[15,56] Natural water deprivation, as is evident from the data presented earlier, causes depletions of both ICF and ECF. The thirst and eventual water intake observed is the sum of the signal from these 2 components.[15,48,53,56]

**Water restriction.** Water restriction can be useful as a motivating stimulus in behavioral studies.[21] Most animals adapt well to once-daily access to water, and this frequency seems to be common for primates in arid natural habitats.[12] Hughes and colleagues[23] reported intakes and weight changes of rats during 2 mo of water restriction in a water-rewarded operant task. Groups of rats were deprived for 7, 14, or 21 h daily. Operant groups received a 1-h test session at the end of this time and then had free water and food in their home cage for the remainder of the scheduled access. Nonoperant groups also were run and simply received water restriction for the same durations. Their weight changes are shown in Table 4. Only the 21-h group showed an initial small weight loss and a modest reduction in weight gain during the study. However, only the 21-h operant group was able to acquire the water-motivated task, indicating that this level of water deprivation has distinct motivational characteristics, compared with 7- or 14-h deprivation. Likewise, Carlton[9] showed that responding for water on interval schedules was a steep function of deprivation time, with a maximum after 23 h. Although the timing and amount of food consumed usually is not measured, the weight gain data given by Hughes and colleagues[23] indicate that dehydration anorexia was minimal. Other studies have shown that water-deprived rats drink and then alternate eating and drinking,[67] so most of the food intake likely occurred during the period of water access in the home cage. Kakolewski and Deaux[25] reported that rats accustomed to a 15-ml ration of water once daily ate 4.2 g dry food during the ensuing hour; this quantity is not as much as their expected total daily intake but is a substantial meal. Therefore, animals seem to be well able to entrain their ingestive responses to these times of availability without evidence of compromised health.[23] Data relating the time course of changes in body fluid parameters during this type of chronic restriction have not been published, but as for acute deprivation, the type and timing of food access would be key variables. Even mice, with their small body size and relatively larger effect of 24-h water deprivation (as discussed earlier), survive severe water restriction schedules,[41] although the ration in the cited report should not be used routinely.

Because many behavioral experiments are performed only 4 or 5 d per week, some investigators allow animals ad libitum access to water over the weekend. This practice allows recovery of food intake, and body weight of these animals shows a 'scalloping' effect. However, whether this pattern is an improvement over restriction on 7 d per week is debatable; primates, including humans, living in chronically water-restricted conditions adapt physiologically and behaviorally to the routine, so breaking that routine may not in fact be beneficial.[12,42]

**Recommendations regarding water deprivation or restriction.** 1) Rats and mice are physiologically equipped to tolerate acute dehydration for periods of as long as 24 h without overt signs of physiologic distress or behavioral abnormalities. 2) Water deprivations in excess of 24 h produce only small additional changes in hydration level but do produce substantial anorexia. Therefore, for most purposes, deprivation in excess of 24 h likely is not necessary. Because most food intake occurs at night, overnight deprivation with food available should produce comparable dehydrating effects to a full 24 h of water deprivation. Deprivation of as long as 72 h is tolerated by rats, with weight

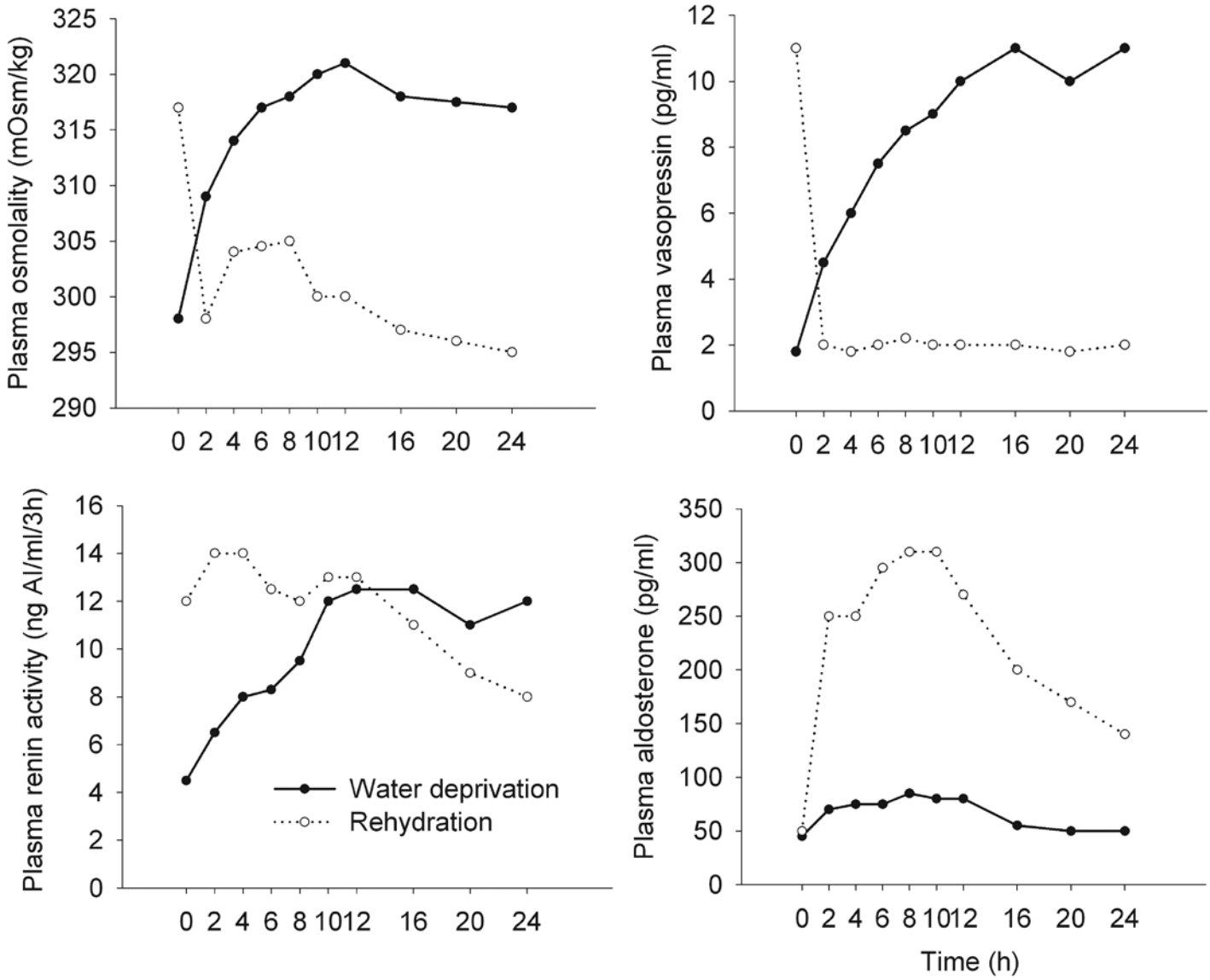


**Figure 4.** Changes in plasma osmolality and renin activity and concentrations of aldosterone and vasopressin during 24-h periods of water deprivation (dark symbols) and rehydration (light symbols) in dogs. Animals were fed once each day at time 0. Figure is redrawn from data in reference 35.

loss in an acceptable range (approximately 11%) and no apparent loss of physical vigor. However, the need for such longer durations should have strong scientific justification. Shorter times may be more suitable for smaller rodents, such as mice, although data are lacking. 3) Because of endogenous circadian rhythms of physiology and behavior, deprivation or restricted access of 24-h duration and periodicity often is particularly well accommodated. 4) Animals adapt well to once-daily water restriction schedules but should be allowed sufficient access time each day to allow them to consume at least some food while water is available and so that established criteria for food intake or weight change are satisfied. Suitable criteria might be that the animal is eating 75% of ad libitum food intake or loses less than 15% weight, but these values are offered only as examples and may differ between species or with different types of food. 5) Water-restricted animals should be weighed regularly and provision made in the protocol for supplemental water if the approved criteria are not met.

## Physiology of Food Deprivation and Restriction

**Energy compartments and expenditure.** The section on normal feeding and drinking introduced some general principles about cycles of food intake and energy storage or mobilization. About 50% to 75% of the energy expenditure under normal laboratory conditions is basal metabolism, the energy used to maintain body temperature and resting biologic processes. The thermoneutral range is the range of environmental temperature under which basal metabolic rate is approximately constant. The *Guide for the Care and Use of Laboratory Animals*[40] has recommended ambient temperature ranges for the maintenance of laboratory species, and these are in part based on published thermoneutral ranges. Although many animals can survive for long periods outside of these ranges, basal metabolism is higher, especially in the cold when extra body heat is lost to the environment. Most animals increase their daily food intake substantially in the cold.[8] Pregnancy and especially lactation are other circumstances of increased energy loss, in this case to the offspring, and these conditions are

**Table 4.** Effect of various durations of chronic water restriction in ovariectomized female Long-Evans rats

| Restriction | Minimum body weight (% initial) | Body weight (% initial) 60 d later |
|---|---|---|
| None | 100 | 120 |
| 7 h/d | 100 | 117 |
| 14 h/d | 98 | 121 |
| 21 h/d | 96 | 114 |

Data are derived from reference 23, Figure 3 A.

associated with increased food intake. Husbandry programs often recommend feeding pregnant rodents a diet of higher energy yield. Physical activity typically accounts for less than 25% of the energy expenditure of sedentary rodents, and this proportion can be increased by regimens of vigorous exercise. Exercising animals do not always increase their food intake, and in this event they maintain leaner body weights than sedentary animals.[14]

To a first approximation and over a suitably long time frame (at least 24 h), any mismatch between energy expended and energy intake results in a reciprocal change in nutrient stores of the body. The primary nutrient store is adipose tissue. During food deprivation, adipose tissue will be the principal source of calories to fuel metabolism. A progressive and adaptive decrease in metabolic rate occurs as the duration of deprivation increases.[30] Therefore, during chronic food restriction, a new equilibrium is established between the reduced intake and the reduced expenditures to achieve stable body weight. Across a wide range of conditions,[29] metabolic rate is proportional to (body weight in kg)$^{0.75}$.

**Physiologic aspects of food deprivation.** The amount of energy stored in adipose tissue varies extensively between individuals of a species, between species, and as a function of environmental conditions, so the physiologic impact of food deprivation will in part depend on initial adiposity. For example, a 30-g mouse with 10% body fat content (3 g) has approximately 27 kcal stored as fat. Mice typically eat 12 to 14 kcal/d; thus this adipose store amounts to 2 d of ad libitum food intake. By the same calculation, a genetically obese mouse weighing 60 g with 55% body fat content (33 g) has approximately 297 kcal stored as fat; even if these mice ate twice as much as lean mice (they do not), this quantity would be equivalent to more than 11 d of stored energy. For a larger animal, a 300-g rat with 10% body fat will have 270 kcal stored, and at 65 kcal/d food intake, this amount is equivalent to 4 d of stores. These oversimplified examples, which among other things do not account for reduced metabolic rate during deprivation, show that larger animals either by species or by fatness are better able to withstand starvation than are smaller animals. Young and growing animals have a smaller percentage of body fat than do adults and thus are more vulnerable during starvation. It follows that any consideration of the physiologic and possibly the psychologic effects of food deprivation must consider the age and initial state or adiposity of the animal.

Armstrong and colleagues[1] reported the effects of food deprivation for as long as 4 d in rats; selected aspects of those data are shown in Table 5. Weight loss occurred as a negatively accelerated function of time, with approximately 7% of initial weight lost occurring in the first 24 h. Some ACUCs use weight loss criteria such as 10% or 15%, which correspond to slightly less than 48 and 72 h of food deprivation, respectively, in rats of this age (approximately 5 mo) and initial weight (approximately 440 g). In the cited study, activity was measured by using a movement detector in the home cage and increased progressively above baseline, particularly during the daytime. Under ad libitum conditions, daytime activity was approximately 25% of total, but by the 4th day of deprivation, it had risen to almost 50%.

Severinsen and Munch measured activity and core temperature of Wistar rats continuously using a small implanted transmitter[58] and obtained a slightly different result. One group of rats was food-deprived for 9 d, whereas another group was fed 25% of the ad libitum ration every morning. Over this time period, the authors reported a 27% loss in body mass in the animals starved for 9 d and a 13% loss in the restricted rats. Core temperature continued to show a circadian rhythmicity, and mean daily temperature decreased by up to 0.4 °C in the restricted group and 1.1 °C in the starved group. This change in temperature was consistent with a previously reported decline in resting metabolic rate of 22% and 31% after the ninth day of restriction or deprivation.[39] In contrast to the result of Armstrong and colleagues[1] mentioned earlier, locomotor activity in the Severinsen and Munch study decreased by approximately 20% during the first day of deprivation and by 34% and 48% of control by the ninth day of restriction or deprivation, respectively. Locomotor activity during deprivation is likely to depend on measurement technique and ambient temperature. Severinsen and Munch[58] used a relatively high ambient temperature of 28 °C, which reduces energy loss by thermal conductance that occurs in proportion to the difference between body and ambient temperatures. Therefore, food deprivation at higher ambient temperature will result in greater metabolic savings and less weight loss than would the same duration of deprivation at low ambient temperature. Studies proposing prolonged food restriction or deprivation should specify and monitor the ambient temperature.

In these examples, after the deprivation period, animals readily refeed and gain back most of the lost weight within a few days (Table 3). Even relatively prolonged food deprivation appears to have no long-term adverse physiologic effects. Nonetheless, investigators should always use the shortest period of deprivation consistent with study objectives. Lastly, as is the case for water deprivation, food deprivation in multiples of 24 h are scientifically the most defensible because circadian variables are minimized; food deprivation for 24 h leads to less than 10% weight loss in most species, and this amount should routinely be acceptable. Longer periods need careful justification and review, including a daily surveillance protocol and end points.

**Physiologic aspects of food restriction.** Food restriction offers a more complex set of issues than total deprivation. Restriction regimens most often are used for behavioral experiments in which a consistent level of motivation is needed from day to day. Animals often are food-deprived for 16 to 22 h and then are tested in a behavioral task in which food or another commodity, such as a drug, serves as a reinforcer. Either immediately after the session or after some delay the animals are fed a free daily ration that complements the amount consumed during the test session. The total amount of food eaten either is held constant or varied slightly depending on whether the animal is above or below a target weight.[9] Most investigators either have developed or follow protocols that use a food ration that has been documented previously to produce stable performance in behavioral tasks. This type of study often takes months or even years; therefore a question that invariably arises is whether either the proposed level of rationing might be less severe, or whether rationing is necessary at all, in order to achieve the scientific objective.

**Table 5.** Effect of various durations of food deprivation on body weight loss, home cage activity, and recovery in Wistar rats

| Duration of food deprivation (h) | Weight loss (% initial) | Activity (% baseline) | Days of refeeding needed to reach 95% of initial weight |
|---|---|---|---|
| 24 | 7 | 106 | <1 |
| 48 | 12 | 112 | 6 |
| 72 | 17 | 116 | 11 |
| 96 | 19.5 | 130 | 8 |

Data are derived from reference 1.

First, let's examine the criteria for maintaining such animals. ACUCs often use a criterion of weight loss (for example, 10% or 15%). Recall from the earlier discussion that these percentage losses correspond to those after 24 to 72 h acute deprivation in rats, recognizing that there may be species and strain differences. Many animals are started in these experiments in young adulthood, but if freely fed from that age, they would probably have continued to grow for some time.

A recent experiment using Sprague-Dawley rats clarified the relationship between long-term rationing and consequences for body weight.[27] Parts of these data are summarized in Table 6. First, in the ad libitum-fed groups, both male and female rats gained weight throughout life. In male rats, this gain was more rapid during the first year, whereas in female rats, it occurred prominently during the second year, possibly as a result of losing ovarian cyclicity and estrogen, a known appetite suppressant. Second, in both male and female rats, the percentage of body fat approximately doubled between young and mid-to-late adulthood. Third, this gain of weight or fat was associated with high blood cholesterol and triglycerides, organ pathologies, loss of estrous cyclicity in more than 50% females by 9 mo of age, and high mortality (only approximately 18% survived for 113 wk).

The most stringent food restriction (48% of initial ad libitum intake) was associated with slow weight gain throughout the study. If this gain was expressed as a percentage of the ad libitum values, the restricted rats appeared to lose weight but only because the ad libitum comparison groups continued to gain weight. In contrast to that of the ad libitum-fed rats, the body fat of the 48% groups remained constant and low throughout the study. These rats also had low blood cholesterol and triglycerides, fewer organ pathologies, sustained estrous (approximately 25% rats cycling even at 2 y), and low mortality (approximately 80% surviving at 113 wk).

Intermediate food restriction (75% of initial ad libitum intake) produced intermediate effects. The report of Keenan and colleagues[27] actually used 2 groups restricted to about this extent, but for simplicity I have discussed only 1. Male rats gained some body weight and fat content during the experiment (mean weight at week 113 was 146% that at week 20), and their body weight remained at a constant proportion (78% to 84%) of ad libitum values. Female rats also gained weight (mean weight at week 113 was 136% that at week 20) to a level comparable to that of males. However, unlike males, female rats maintained body fat expressed as percentage of weight, but their weight as percentage of ad libitum values fell due to gain by the ad libitum groups.

Keenan and colleagues[26] have advocated the use of partial restriction to 75% of ad libitum intake in long-term studies that require maintaining a healthy population for years (including, for example, toxicologic or behavioral work). Their recent results[27] show that ad libitum feeding of chow, a relatively low-fat diet, to rats over a lifetime produces body fat levels that in humans would be considered obese with attendant metabolic problems. In adult humans and rodents, changes in weight are reflected as a proportional change in body mass index [BMI], so the 74% increase in BMI between 33 and 113 wk in female rats [Table 6], translated to human scales, would certainly be alarming to a physician. A 75% ration slows weight gain and allows a reasonable body fat content. A 50% ration maintains a lean but very healthy rat. Such restricted feeding regimens may not be feasible for use in routine animal husbandry and may not be necessary in the majority of studies, which are relatively short term, but these data show clearly that using ad libitum-fed animals as a 'gold standard' against which to gauge the effects of food restriction in prolonged studies is not appropriate. The gains shown during aging in the 75% group (Table 6) seem to offer more reasonable standards for growth in rats and may be suitable for mice.

A final point on this topic is obvious individual variation. For example, considerable ranges in body weight at 7 wk of age are shown in the footnote to Table 6. Therefore, expressing target weights as the percentage of a group mean may be inappropriate: for an animal that begins restriction at 10% above the mean weight, a weight restriction to 20% below the mean represents a restriction to 30% for that animal. Conversely, for an animal that starts at 10% below the mean, a weight restriction to 20% below the mean represents an individual restriction of only 10%. Reliance on mean growth charts may introduce unacceptable individual variability in actual weight loss (a 10% to 30% range in this example) and, most likely, in the resultant motivational state. To minimize but probably not eliminate this variability, I advocate restriction regimens that target a weight gain trajectory based on the initial weight of each individual. Sensible targets in rats might be 2 g/wk in males and 1 g/wk in females. These levels correspond to the average weight gains between weeks 20 and 113 in the 75% groups from Table 6.

**Psychologic aspects of food restriction.** In addition to the physiologic aspects of food restriction, ACUCs often consider the psychologic aspect. This task is extremely difficult and subjective because in the absence of verbal reports from the animals, humans often anthropomorphize. The field of food-motivated behaviors in animals gives important insights into their subjective states and so may be an objective lens through which we can examine questions about psychologic aspects of food deprivation and restriction. For example, although humans can easily discriminate between 2 h and 22 h of food deprivation, painstaking training is necessary to get rats to reach a criterion of only 80% correct.[24] Therefore, asking animals "How hungry do you feel?" in this way will not produce a quick or infallible answer.

Performance on interval schedules of reinforcement is quite sensitive to deprivation level. Clark[10] maintained rats at slightly less than 85% of free feeding by feeding them once daily with a calculated ration of dog chow. The rats first were trained by using variable-interval schedules (1, 2, or 3 min) of food reinforcement. In this type of schedule, food is delivered contingent on a lever

**Table 6.** Effect of chronic dietary restriction on mean body weight (g and % of ad libitum) and body fat (% body weight) in Sprague-Dawley rats

| Chronologic age (weeks) | Male rats | | | Female rats | | |
|---|---|---|---|---|---|---|
| | Ad libitum | ~75%, 24 g/d | ~48%, 14.5 g/d | Ad libitum | ~75%, 17 g/d | ~48% 11 g/d |
| Body weight | | | | | | |
| 20 | 491 | 409 (83%) | 276 (56%) | 272 | 239 (88%) | 180 (66%) |
| 33 | 614 | 482 (78%) | 306 (50%) | 346 | 260 (75%) | 184 (53%) |
| 60 | 751 | 587 (78%) | 353 (47%) | 412 | 295 (72%) | 212 (51%) |
| 113 | 712[a] | 597 (84%) | 345 (48%) | 603 | 324 (54%) | 215 (36%) |
| Fat content | | | | | | |
| 20 | 17.4 | 16.4 | 9.5 | 19.9 | 12.4 | 6.4 |
| 33 | 24.6 | 18.4 | 9.1 | 24.4 | 12.2 | 4.0 |
| 60 | 34.7 | 26.7 | 8.6 | 32.7 | 14.7 | 4.8 |
| 113 | 36.5 | 27.0 | 8.1 | 42.2 | 13.2 | 5.7 |

Values derived from reference 27, Tables 2 and 3 A. Rats started the study at 7 wk of age, when males weighed 172 to 266 g and females weighed 134 to 213 g. They were euthanized 13, 26, 53, or 106 wk later. Throughout the study, rats were fed Purina Chow either ad libitum or once daily with the amounts indicated (in g/d and as % of the ad libitum group at the start of the study). Mean body weights (in g, and as % of that of corresponding ad libitum group) have been rounded to the nearest integer.
[a]Mean weight loss at 113 weeks may be an artifact of high mortality.

press but only after an average interval. This scenario yields constant rates of responding, but because the actual delivery intervals vary unpredictably around the mean, the animals cannot predict the exact time until the next food reinforcer. When this behavior had stabilized, rats were tested at various times (1 to 23 h) after the previous meal of dog chow. The results, redrawn in Figure 5, show that for equivalent elapsed time since the last meal, rats responded at higher rates on 1-min compared with 3-min variable-interval schedules. In addition, response rate increased nonlinearly with duration since last fed, but fractional changes were identical at the 2 ratios.[10] Important for the present purposes, the rate after a 23-h delay was about double the rate after a 3-h delay. Therefore, if the relative rate of responding on a variable-interval schedule can be interpreted as a psychologic manifestation of hunger, then animals are about twice as hungry after 23 h as after 3 h without food. This proposition may seem controversial, but it is objectively based.

Corroborating data were obtained by Reese and Hogenson,[49] who examined pigeons pecking an illuminated key under a fixed-ratio schedule in which each peck produced a 4-s access to a tray of grain. At each session, pigeons were allowed to feed until satiation, which was defined as no responses for 30 min. The experimental variables were time of deprivation and associated weight loss at the time of the test for satiation. Their first result was that the rate of key pecking, and presumably eating, was almost always all-or-none; whenever pigeons were eating, median response rate was 4/min. Therefore, pigeons eating a uniform food show relatively abrupt satiation as manifested by transition from uniform responding to no responding; this situation is also the case in rats freely eating from a jar of food.[31] However, pigeons did not respond at all until they were food-deprived for approximately 35 h or were approximately 85% of the free-feeding weight.[49] Most subsequent studies in pigeons have used 85% weight as a standard ceiling body weight, with a level of food restriction consistent with obtaining this weight, thereby maintaining consistent behavioral performance. Studies in rodents have used comparable levels of weight loss to obtain stable performance.

It has been recommended that animals should work for palatable food treats in this type of study, as an alternative to food restriction.[40] However, although many animals do readily consume treats, such as cereal or candy, the evidence that they are highly motivated to do that is sparse. For example, using a progressive ratio schedule in which each pellet costs more than the previous pellet, nondeprived rats worked an average of 105 presses for a total of 6 sweet food pellets whereas rats deprived of food for 24-h worked an average of 600 presses for 15 pellets.[60] It follows that rats would not sustain operant performance through a standard session using palatable treats without some level of restriction or weight loss. Another functional difference between restriction and nonrestriction protocols was reported by Barbano and Cador,[3] who found that food-restricted rats developed the well-known behavior of heightened activity in anticipation of feeding, but nondeprived (palatable food-fed) rats did not.

Given that no completely uniform or agreed-upon levels of restriction exist for specific tasks, investigators tend to use a level of restriction that is more than adequate to ensure that the animals will reliably perform the tasks required to answer the scientific questions. Therefore, even if the minimum amount of deprivation needed to sustain a task were known for a range of tasks, maintaining subjects at that minimum would make performance less reliable, data would inevitably be wasted, and more animal-days would be necessary to complete the study. Initial weight restrictions to 85% of free-feeding, followed by an increment appropriate for the species, sex, and strain, likely will be adequate to ensure strong performance in most behavioral tasks and still be consistent with an animal that is at least as healthy as ad libitum-fed (and overweight) counterparts. With justification, even lower target weights may be acceptable.

To conclude this section: animals on food restriction regimens must be given food that is sufficiently high in quality to ensure they do not become deficient in any specific nutrient, such as protein or micronutrients. The lower the target weight or food ration, the more critical this consideration becomes. If treats are used as supplements or reinforcers, they should contain protein and micronutrients.

**Recommendations regarding food deprivation or restriction.** 1) Most species are physiologically equipped to tolerate acute withholding of food for periods of as long as 24 h without notewor-

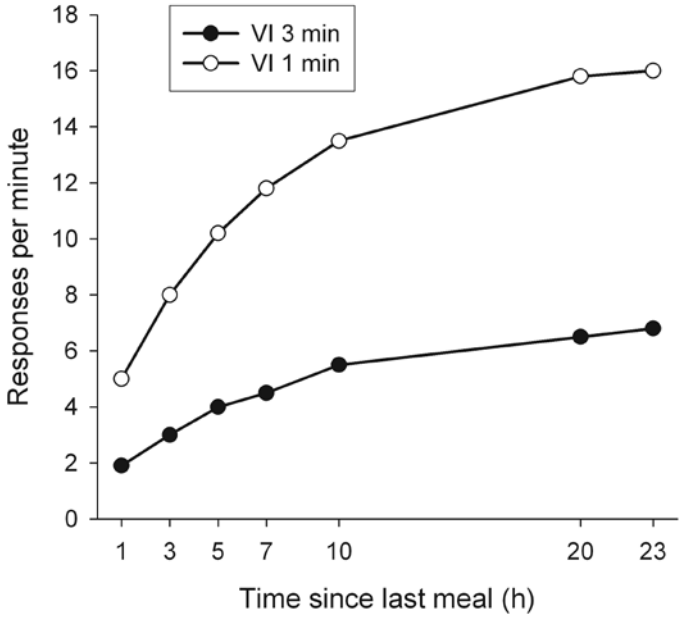


**Figure 5.** Responding of rats for food reinforcement during the early portion of variable-interval (VI) sessions. Only 1- and 3-min schedules are shown for simplicity; 2-min data were intermediate. The response rate was higher overall on the shorter-interval schedule, but the degree changes with time, because the last meals were similar in the 2 conditions. Figure is redrawn from data in reference 10.

thy signs of physiologic or apparent psychologic distress. 2) Food deprivation in excess of 24 h engages a number of physiologic strategies for saving energy; therefore weight loss is a negatively accelerated function with days of deprivation. For many purposes, deprivation in excess of 24 h likely is unnecessary. With scientific justification, deprivation of as long as 72 h is acceptable in rats, producing weight loss in an acceptable range (approximately 15%) without lethargy. Shorter times may be more suitable to achieve comparable physiologic or behavioral effects in mice. 3) Because of endogenous diurnal rhythms of physiology and behavior, animals often accommodate deprivation or restricted access in multiples of 24 h in duration and periodicity particularly well. 4) Animals adapt well to once-daily food restriction schedules. Water should be available either continuously or (at a minimum) for the entire period provided for consumption of the daily food ration. The target intake or weight for such restriction will depend in part on the species and the objective of the study, but 85% is common for rats and birds in behavioral studies. Weight reductions much less than this may not be sufficient to achieve stable behavioral performance. Mice may require and tolerate comparable target weights, although extensive data are not available. 5) Using palatable treats instead of deprivation often does not produce sufficient motivation for behavioral studies. Although treats may be used for environmental enrichment, they should be part of the overall nutritional program for the animals and the study.

## Summary and Conclusions

Because food and fluid deprivation or restriction has occurred in the evolutionary past of all extant terrestrial species, animals are physiologically well equipped to deal with such privations. We have reviewed evidence that shows that after 12 to 24 h without access, animals efficiently reduce further fluid or energy losses by a combination of behavioral and physiologic adjustments. These presumably minimize the additional physiologic or psychologic stress of deprivation. Animals have endogenous nycthemeral rhythms that make them particularly adaptable to once-daily occurrences, such as food or water access. Therefore, deprivations of 24 h and once-per-day restricted-access schedules seem to be minimally stressful by the parameters of normal behavior and appearance. Longer periods of acute deprivation or chronic restriction may be acceptable procedures, but ACUCs must ask investigators to implement suitable monitoring protocols, such as routine weighing and target weights. In the case of chronic food restriction, using a fraction of the weight of age-matched free-fed animals as a target may be inappropriate because ad libitum-fed animals become obese and have shortened life expectancy. Species, age and strain-specific target growth rates may be more appropriate but are not always available. Data from rats have been presented in this review because they are available, but empirical normative data from other species, especially mice, are lacking.

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