

Food and Water Restriction Protocols: Physiological and Behavioral Considerations

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Abstract | Food and water restriction protocols are common in animal research, yet they often elicit discussion and controversy among institutional animal care and use committee members who review them. Determining a single standard by which all restriction protocols can be evaluated or performed may not be realistic. However, information about the physiologic and behavioral impact of food and water restriction can provide a basis for making rational judgments about these issues in general. This review will discuss the physiologic and behavioral consequences of food or water deprivation periods of 24 h or less and of chronic restriction schedules, with special reference to protocols that use food or water restriction as a motivational tool for behavioral training.

Food and water restriction protocols are common in animal research, yet often elicit discussion and controversy among institutional animal care and use committee (IACUC) members who review them. Three issues are particularly important in the evaluation of restriction protocols. First, the need for and the necessary degree of restriction must be determined. Second, the potential adverse consequences that the animal will experience as a result of the restriction must be assessed. Third, the proposed maintenance care must be evaluated in terms of ensuring animal well-being yet allowing successful completion of the research goals. Determining a single standard by which all restriction protocols can be evaluated or performed may not be realistic. However, information about the physiologic and behavioral impact of food and water restriction can provide a basis for making rational judgments about these issues in general.

A conceptual distinction can be drawn between the terms “restriction” and “deprivation.” Deprivation implies the total denial of food or water and generally is described in terms of the interval during which food or water is withheld from the animal. In contrast, restriction implies a limitation on ad libitum intake, as opposed to total denial. Restriction is typically described in terms of either the amount of food or water provided on a daily basis or the amount of time each day that the animal is given access to food or water. Although the terms restriction and deprivation overlap conceptually and are often used interchangeably, these procedures may affect the experimental animal in different ways. In many restriction schedules, for example, animals have the opportunity to eat or drink to satiation each day, but the number of opportunities may be limited to one per day. Because relatively few studies subject animals to multiple days of total deprivation, this discussion will focus primarily on the physiologic and behavioral consequences of deprivation periods of 24 h or less and of chronic restriction schedules.

Water Restriction

Assessing the impact of water restriction on an animal's health and well-being requires an understanding of the physiologic mechanisms that regulate thirst, fluid intake, and hydrational homeostasis. The two primary physiologic stimuli that elicit thirst under normal conditions are cellular dehydration and hypovolemia (1–3). When an animal becomes dehydrated due to

inadequate water intake or excessive renal or evaporative losses, the osmolality of extracellular fluids increases, and the volume of fluid in the circulation is reduced. Cellular dehydration then develops because the extracellular osmotic gradient draws water out of cells. Cellular dehydration also can develop due to excessive intake of solutes such as sodium chloride. Because of these relationships, plasma osmolality and plasma sodium concentrations are good indirect indices of cellular dehydration. Drinking lowers the concentration of extracellular solutes, thereby reducing the extracellular osmotic gradient and allowing water to move back into the cells and to restore intracellular fluid volume. Hypovolemic thirst develops when fluid leaves the circulation, as occurs during simple dehydration and during clinical conditions such as hemorrhage. In the absence of vascular leakage or blood loss, hypovolemia can be assessed by measuring hematocrit or plasma protein values, because these values increase when water is lost from the vascular compartment. Extracellular solutes are necessary for osmotic retention of fluid in the interstitial and vascular compartments. To restore plasma volume deficits, an animal may need to ingest solutes as well as water, because otherwise, most of the fluid that is consumed will move osmotically into the intracellular compartment rather than remain in the extracellular compartment. In addition to cellular dehydration and hypovolemia, the hormone angiotensin is a stimulus for thirst under some specific physiologic conditions (e.g., during hypotension), but this compound probably does not contribute significantly to thirst associated with water deprivation (4).

Dehydration that develops during water deprivation is associated with fluid loss from both intracellular and vascular compartments. A period of 24 h without water causes increased plasma osmolality and plasma sodium concentrations, reflecting changes in the cellular pool, and increased hematocrit and plasma protein values, reflecting changes in the vascular pool (Table 1). The proportion of postdeprivation drinking that occurs in response to cellular dehydration versus hypovolemia varies across species. In rats, dogs, and monkeys, which are the three species that have been studied most extensively, cellular dehydration elicits approximately 65%, 75%, and 85%, respectively, of the total water consumption that occurs after 24 h of water deprivation and thereby accounts for a large proportion of the total stimulus for thirst (1). The threshold for thirst in response to cellular dehydration can be estimated as the plasma osmolality associated with the initiation of drinking after sodium infusion; this threshold value can be compared with the plasma osmolality measured after 24 h of water deprivation (Table 2). The

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Table 1. Changes in body fluid variables produced by water deprivation in four species^a

Species	Osmolality ^b		Sodium ^b		Hematocrit ^b		Plasma Protein ^b	
	NonDeprived	Deprived	NonDeprived	Deprived	NonDeprived	Deprived	NonDeprived	Deprived
Rat (n = 15)	299.6 ± 0.5	306.1 ± 0.6*	138.9 ± 0.5	139.9 ± 0.5	42.2 ± 0.7	46.2 ± 0.6*	7.5 ± 0.2	7.8 ± 0.1*
Dog (n = 8)	298.5 ± 0.7	310.3 ± 0.9*	147.3 ± 0.7	153.7 ± 0.7*	42.4 ± 2.0	47.3 ± 2.1*	5.5 ± 0.1	6.1 ± 0.1*
Rhesus (n = 5)	297.8 ± 2.4	311.0 ± 4.5*	143.0 ± 1.7	149.2 ± 2.0*	36.6 ± 1.9	35.8 ± 1.1	7.1 ± 0.2	7.4 ± 0.2*
Human (n = 5)	282.4 ± 2.2	289.8 ± 1.8*	140.4 ± 0.7	143.3 ± 0.6*	47.2 ± 1.8	48.2 ± 2.3	7.3 ± 0.2	7.7 ± 0.2*

^aRats were subjected to 21 h of water deprivation, whereas other species were subjected to 24 h of water deprivation. Modified from references (1, 82).

^bUnits: osmolality, mOsm/kg H₂O; sodium, mEq/liter; hematocrit, %; plasma protein, g%. All values are the mean ± S.E.M.

**P* < 0.01 relative to values measured in the non-deprived state.

Table 2. Plasma osmolality at drinking threshold and after water deprivation

Species	% Increase in Plasma Osmolality		
	At the NaCl-Induced Drinking Threshold ^a	After 21 or 24 H of Water Deprivation ^b	Ratio
Rat	1.6%	2.2%	1.4
Dog	2.9%	4.0%	1.4
Rhesus	2.3%	4.4% (5.8%)	1.9 (2.5)
Human	1.2%	3.0%	2.5

^a The values are the percentage changes in osmolality measured when spontaneous drinking develops after infusion of a hypertonic NaCl solution. References: rat, (83); dog, (84); Rhesus monkey, (82); human (84).

^b The values are the percentage changes in osmolality measured after water deprivation, as calculated from the data presented in Table 1. Rats were deprived of water for 21 h, whereas other species were deprived for 24 h. The additional value provided in parenthesis for Rhesus macaques is based on data presented in (82).

plasma osmolality after 24 h of water deprivation exceeds the osmolal threshold for drinking, as would be expected given that thirst would be present at this time. However, the precise osmolal threshold for drinking varies across species, being lowest in man and highest in dogs (Table 2).

Cellular dehydration and hypovolemia not only stimulate thirst, but they also initiate renal mechanisms for fluid conservation. Animals do not become progressively twice as dehydrated during a second 12-h deprivation period as during the initial 12 h because homeostatic mechanisms for fluid conservation function to retard water loss. In rats, which are particularly efficient at renal water conservation, deprivation periods of either 12 or 24 h do not result in markedly different amounts of compensatory intake when water is made available, although intake is substantially greater after longer periods of deprivation (5, 6). The degree of dehydration that develops after 24 h without water varies across species (Table 2). In humans, the change in osmolality that occurs after 24 h of water deprivation is 2.5 times greater than the change necessary to cause thirst. However, the difference between these values is less in other species (Table 2). These relationships imply that after 24 h without water, humans become more dehydrated relative to their thirst threshold than do other species. These species differences suggest that humans may overestimate how thirsty an animal is likely to become after a given period without water.

Nonhydrational factors also influence daily fluid intake (Table 3). Ad libitum fluid intake often exceeds hydrational needs, can be strongly influenced by habit or learning, can show characteristics of a rhythm entrained with feeding, and can occur in the absence of fluid deficits or hormonal signals (4). Humans and animals will drink solely in response to conditions such as eating a meal, a dry mouth, or the ready availability of a palatable beverage, and fluid intake may be reduced if gaining access to the beverage is difficult or even inconvenient. Therefore, fluid consumption is influenced by thirst (which in turn reflects hydrational status), pharyngeal factors, palatability and availability of a beverage, and even learning or habit.

Table 3. Factors that influence drinking and eating

Drinking	Eating
Fluid deficit (thirst) (Cellular dehydration) (Hypovolemia)	Metabolic need (hunger) Lack of satiety
Peripheral factors (e.g., dry mouth)	Peripheral factors (e.g., aroma)
Variety and palatability	Variety and palatability
Availability/ease of access	Availability/ease of access
Prandial factors	
Habit	Habit

Because of nonhydrational influences, ad libitum intake of fluids may not accurately reflect hydrational needs (4). One approach to estimating hydrational requirements is to determine how much animals drink if their water is very unpalatable or if obtaining water requires satisfactory completion of a task. In one study, rats that were given unpalatable quinine-adulterated water initially drank very little and lost as much as 20% of their initial body weights (7). They gradually resumed drinking but continued to drink less than rats given tap water. Despite these reduced intakes, their consumption was still sufficient to maintain growth at a normal rate. Furthermore, rats maintained on unpalatable water adjusted their intake to compensate for hydrational challenges. For example, when salt was added to the diet, they increased their consumption, and when given a vegetable diet with a high water content, they drank less. In this study, rats given water containing 0.1% quinine drank about 20 mL per day. However, this level is not necessarily equivalent to minimal adequate requirements, because spontaneous consumption changes inversely with the concentration of quinine (8). These observations indicate that animals typically consume more fluid than is physiologically necessary, because they will reduce their intakes and thereby presumably tolerate some degree of dehydration rather than drink an unpalatable fluid, yet the animals remain responsive to their physiologic needs and will increase their consumption when necessary.

In other studies, rats were taught to press a bar for infusion of water through an indwelling intravenous catheter (9, 10). The rats adjusted their rate of bar pressing based on the amount of water delivered after each press, maintaining average daily water intakes of about 15 mL. Body weights decreased to about 80% of initial values. Most of the rats were oliguric and produced a concentrated urine, indicating renal conservation of water. Water consumption occurred primarily at night and was associated with eating. Intakes increased in response to stimuli such as heat or an increase in the salt content of the food. When the rats were eventually given oral access to water, they drank approximately 20 mL during the first hour, indicating a subjective state of thirst despite the neglected opportunity to behaviorally ameliorate the dehydration by pressing the bar.

These findings indicate that rats can maintain homeostatic balance even when they consume significantly less than their daily ad libitum intake of water. Because they could have consumed more water in these studies, their dehydration was presumably not excessively uncomfortable. Fluid consumption under these conditions was roughly equivalent to needs as determined by clinical formulas for calculating fluid maintenance requirements, which are often estimated to be approximately 50 mL/kg daily (11, 12). According to that formula, a 300-g rat would require approximately 15 mL water per day. Fluid homeostasis is possible with even less water than this; the minimum amount of water intake needed for long-term homeostasis would be equivalent to the daily urinary, fecal, and evaporative losses in an animal that was producing a maximally concentrated urine. Within that constraint, animals will be thirsty and will drink if an acceptable fluid is available but are nonetheless capable of maintaining homeostatic balance.

In developing a water restriction schedule, consideration should be given not only to the total amount of daily water intake but also to the schedule of water availability. Drinking ideally should occur when food is also available. The concurrent availability of water and food incurs two benefits. First, fluid intake promotes food intake, thereby reducing the likelihood of dehydration-related anorexia. Second, the consumption of food in association with water allows animals to consume solutes that will help retain water in the circulation, correct volume deficits, and avoid excessive hemodilution that will cause urinary excretion of the ingested water. Another consideration in deciding how to supply water to animals on restriction schedules is determining the amount of time necessary for adequate daily water consumption. Different species vary considerably in how much and how fast they drink after deprivation. Dogs, for example, drink a large amount very rapidly and then stop, whereas rats drink small amounts relatively frequently (1). However, despite different patterns of drinking, most of the water consumption that will occur during the first hour of access actually takes place during the initial 10 to 30 min (1, 13–15). This amount of drinking also reverses the hematologic changes that develop with water deprivation (1, 13). Plasma osmolality and protein values are high after 24 h of water deprivation but return to normal levels within about 20 min after drinking (1, 14). Thirst also resolves very quickly after access to water. Humans who undergo 24 h of water deprivation report that they are initially very thirsty but become satiated within 5 min of drinking (1). Therefore, relatively brief periods of fluid access are sufficient to alleviate both the thirst and hydrational deficits that develop over 24 h.

In summary, the restricted availability of water for intervals of up to 24 h causes the sensation of thirst, but even chronic restriction schedules do not cause physiologic impairment, assuming that the animals are adapted to the restriction schedule and receive enough water to replenish daily losses. Daily clinical examination and a comprehensive postmortem evaluation revealed no abnormalities in rats that were chronically

Table 4. Regulation of fluid and energy balance

Fluid balance	Energy balance
Stimuli for drinking (thirst)	Stimuli for eating (hunger vs satiety)
Cellular dehydration	Post-absorptive metabolic signals
Hypovolemia	Post-prandial metabolic signals
Specific measures	Specific measures
Serum sodium concentrations	Body weight
Osmolality	Food intake
Plasma protein concentrations	
Hematocrit	
Excess intake is excreted	Excess intake is stored

maintained on a 21-h restriction schedule (16). Chronic water restriction is generally not associated with marked adrenal activation (17–19). Indeed, glucocorticoid elevations during dehydration might cause diuretic effects that would counter fluid conservation mechanisms. Limited availability of water is a common homeostatic challenge, and animals are well adapted physiologically to accommodate this situation. In natural settings, some animals may travel to watering sites only once a day, and in the interim, homeostatic mechanisms automatically regulate their urine output in accordance with their current hydrational state.

Food Restriction

The primary physiologic stimuli that elicit thirst are clearly identifiable as cellular dehydration and hypovolemia (Table 4). However, a similarly concise and generally accepted list of stimuli that elicit hunger under normal conditions or that regulate how much food will be consumed cannot be compiled. The size of the previous meal, rather than the postprandial interval, is more important as a determinant of when the next meal will occur (20), suggesting a direct relationship between the number of calories ingested and suppression of the desire to eat (21). Eating a meal elicits physiologic processes that store ingested nutrients, and this metabolic state is accompanied by feelings of satiety. The physiologic signals that contribute to satiety include processes such as gastric distension, increases in insulin secretion, and metabolic processes in the liver. After consuming and assimilating nutrients, the animal gradually enters a postabsorptive metabolic phase during which stored calories are mobilized to meet metabolic needs. This phase is accompanied by the eventual onset of hunger. However, the specific metabolic parameters that trigger hunger and satiety and regulate food intake remain conjectural. Some experimental stimuli (e.g., antimetabolic drugs or large doses of insulin) can elicit eating in satiated animals, but this response is usually associated with severe hypoglycemia (22), which is rare in normal animals. Such models may reflect pathophysiologic emergencies rather than normal physiologic stimuli for hunger and feeding (22). Identifying stimuli that evoke feeding in satiated animals has been difficult, but a relatively large number of physiologic factors cause hungry animals to stop eating (23), leading to the suggestion that food intake may be regulated less by hunger than by satiety (22).

The incomplete understanding of how hunger and satiety are regulated complicates assessment of the impact of food restriction. Specific physiologic parameters that define or predict metabolic deficiency or hunger have not been identified. Therefore, metabolic needs are generally estimated based on body weight and food intake, as compared either to an animal's own baseline values or to values obtained from control animals. However, individual animals vary tremendously in food intake and in their adult body weights. For example, the daily food intake

Table 5. Food restriction, longevity, and disease incidence in Wistar rats^a

Food availability	Number of rats	Survival at 30 months (%)	Non-neoplastic disease (%)	Any neoplasia (%)	Malignant neoplasia (%)
Ad libitum	300	38	51	83	37
Restricted ^b	200	72	7	68	16
<i>P</i> value ^c	NA	< 0.001	< 0.001	0.021	0.001

^aAdapted from (34).

^bRats maintained on dietary restriction were fed 80% of the amount consumed by rats maintained on ad libitum access to food.

^cProbability values were determined by comparing proportions of animals reported in each category (34) by using a χ^2 test.

of adult male Sprague-Dawley rats can vary from 21 to 32 g per day, and the body weights of genetically identical 12-month-old B6C3F1 mice can range from 30 to 48 g (24). An additional complication is that food intakes and caloric needs may vary with strain, age, housing conditions, physiologic state, the opportunity to exercise, and other characteristics of the animal or the environment (25). The value of food intake and body weight as indices of metabolic need is further complicated by overeating and storage of calories. When animals consume more calories than are necessary to meet metabolic requirements, the extra calories are stored as fat deposits, even if the animal is already obese. In contrast, renal mechanisms not only conserve fluids when animals become dehydrated, but they also excrete, rather than store, excess water. Homeostatic mechanisms generally prevent animals from becoming overhydrated but are less effective at preventing obesity. Knowing an animal's body weight or ad libitum food intake therefore provides only limited information about its minimal caloric requirements.

Another problem with using food intake or body weight as an index of caloric needs is that nonmetabolic factors significantly influence baseline food intake and body weights (26–28). Rats with ad libitum access to food maintain greatly different body weights depending on the palatability and the accessibility of their diet (26, 29, 30). For example, rats fed a quinine-adulterated diet reduced their food intake and maintained body weights at about 80% of control levels, yet the animals appeared healthy, exhibited normal estrus cycles, and grew at the same rate as did control rats (26). In contrast, rats fed a palatable high-fat diet ate more and maintained higher body weights than rats fed a standard diet. Rats on either palatable or unpalatable diets both adjusted their food intake to maintain their respective body weights in response to changing physiologic demands. For example, if the animals were kept in a cold environment, they would increase their food intake enough to maintain the same body weight. Moreover, rats maintained on normal, unpalatable, or highly palatable diets all maintained the same lean body weight; body weight differences associated with varying food palatability were entirely due to corresponding gains and losses in adipose tissue mass (26).

Caloric needs or minimal body weight can be assessed by requiring the animal to work for all of its food under various reinforcement schedules (29, 31, 32). Such studies show that as the work requirement increases, food intake decreases. In one study, food intake at the highest work requirement was about 12 g per day, or 50% of control intakes, and was associated with a 10% decrease from initial body weights (29). Rats trained to press a bar to receive a liquid diet through an indwelling intravenous catheter gradually lost approximately 30% of their initial body weights, but they then maintained and defended that weight by consuming approximately 32 kcal/day (equivalent to approximately 10 g of rodent chow; 32). Although the required task was simple, these rats apparently were not motivated to obtain additional calories in the absence of oral stimuli associated with eating. However, if the caloric density of the diet was altered, the rats pressed the bar more or less often to appropriately compensate for the change. Therefore, animals may spontaneously maintain surprisingly low body weights despite the opportunity

to behaviorally increase caloric intake.

These studies demonstrate that food intake is influenced greatly by non-metabolic factors such as palatability and the ease of acquiring food (Table 3). Under ad libitum conditions, rats clearly eat more food than is required to meet metabolic needs, thereby complicating the determination of minimal nutritional requirements and the assessment of how much food restriction can be imposed without causing severe stress or impaired health. Studies that use food restriction as a motivator often maintain animals at 80 to 85% of their ad libitum or control body weights (33). A relatively large and remarkably consistent group of studies indicates that restricting food intake increases longevity and improves health profiles in all species tested to date (34–37). Rats and mice on dietary restriction generally live up to 50% longer than do animals on ad libitum food intake (34). This increase in longevity is roughly proportional to the degree of caloric restriction. Calorie-restricted animals also develop fewer age-related diseases and fewer spontaneous neoplasms (Table 5). These findings suggest that a thinner animal may be a healthier animal and that estimates of the need for food and of normal body weight can be inflated, prompting some researchers to suggest that ad libitum feeding should be abandoned as the standard way to maintain animals used in aging research, toxicity testing, and carcinogenicity research (24, 35). This consideration may apply to all or most types of research. Many laboratory animal facilities routinely limit the availability of food for a variety of species, including dogs, rabbits, and primates, to prevent obesity, and food restriction is a common veterinary recommendation for pet animals. Overfeeding can even be viewed as malnutrition (24). In general, laboratory rodents may receive ad libitum access to food not because of presumed necessary or beneficial effects of uncontrolled consumption but primarily because of the convenience and cost-effectiveness of simply filling the food container on a regular basis.

Although food restriction may not be harmful, an important related question is whether moderate food restriction is stressful. The answer to this question depends on what is meant by stress. Sympathetic activation and moderate elevations in plasma glucocorticoid levels develop in rats that undergo a 24-h period without food (19, 38–40). However, the magnitude of these elevations are less than those associated with other stressors (41, 42). As opposed to viewing adrenocortical responses to food restriction as indicative of stress, an alternative perspective is to recognize that these changes are adaptive homeostatic metabolic responses to limited food availability (37). Glucocorticoids promote gluconeogenesis and reduce glucose utilization by peripheral tissues, thereby protecting the metabolic needs of glucose-dependent tissues such as the brain. In addition, sympathetic activation promotes improved glucose utilization. Similarly, rats on restricted diets maintain decreased insulin levels (37, 38, 43), presumably to promote glucose utilization rather than storage. However, after 48 h of food deprivation, glucocorticoid levels increase markedly, and their circadian rhythmicity deteriorates (39). These deviations suggest that a deprivation period that is longer than 24 h is associated with increasing metabolic stress and perhaps with the exacerbation of psychological stress.

Another consideration that is relevant to the stress associated with chronic food restriction is that body weights are continuously below normal (44). In contrast, animals maintained on water restriction schedules can generally restore fluid deficits at least daily. Low body weight may provide a long-term stimulus for increasing food intake, but even rats maintained at reduced body weights feed in bouts when given access to food. In one study, for example, rats that were given access to food for 30, 60, 90, or 120 min during the light period all ate about 10 to 12 g, regardless of the length of the access period, and the body weights of all groups decreased to about 85% of initial values (15). This amount of food intake is likely to reflect a full stomach. Therefore, food-restricted rats probably experience periodic relief from the feelings of hunger associated with an empty stomach, despite an underlying persistent motivation to consume more food and thereby to restore *ad libitum* body weights.

The psychologic impact of chronic food restriction is difficult to assess in animals. Some human situations may be somewhat relevant, although truly analogous situations may not be available. Many people live under a self-imposed restriction of food intake (often to about 70% of maintenance requirements), usually for purposes of weight loss or weight maintenance (45, 46). Dieting is not generally associated with adverse psychological consequences (45, 47–49), although mild impairments in cognitive performance have been reported (46, 48). Dieters may experience hunger, the desire to eat more, and preoccupation with food and eating, but these feelings are not equivalent to stress or depression. A situation that may be more similar to that of food-restricted laboratory animals is the experience of soldiers who participate in field exercises. In one study, soldiers on a rigorous 30-day field exercise were given balanced diets that were energy-deficient by 1000 calories (50). The mood, reaction times, and symptoms of calorie-restricted soldiers were no different from those of soldiers on calorie-replete diets. Calorie restriction did not worsen the overall experience significantly, and soldiers on calorie-replete or calorie-restricted diets showed equivalent improvements in mood and symptoms when the exercise was over. In another study, altered levels of cortisol and other hormones levels were viewed as indicative of regulatory changes in energy requirements and glucose metabolism rather than as a generalized stress response (51). In general, food restriction *per se* does not appear to be highly stressful in humans, although it may greatly increase preoccupation with food and eating.

Severe food restriction or total deprivation for several days can be associated with the development of gastric ulcers in rats, particularly if a running wheel is present or if a stressor such as restraint or cold exposure is applied (52–55). A demanding behavioral requirement also could act as a stressor. However, ulcer formation generally requires more severe food restriction than either behavioral reinforcement schedules or the self-imposed limits observed in rats maintained on unpalatable food or intravenous self-feeding. Post-mortem evaluation for gastric ulcers in food-restricted rats may be useful in assessing whether this is a problem in specific experiments. If ulcers develop in rats maintained on specific food restriction schedules, their occurrence can perhaps be reduced by providing food in multiple small meals rather than in one large daily meal.

Several factors influence the design of food restriction schedules. The two basic approaches are fixed-duration schedules, in which animals are given a fixed period of time to consume as much as they want, and fixed-amount schedules, in which animals are given a predetermined amount of food in one or two daily allotments. In fixed-duration designs, an important question is how much time the animals need to ingest enough food to support stable body weights. As mentioned above, rats given access to food for 30, 60, 90, or 120 min all ate about 10 to 12 g, regardless of the length of the access period (15). This finding

suggests that food intake could be controlled by allowing the animals access to food for 1 h per day (56) or by providing animals with a measured amount of food each day (33). For example, if the average food pellet weighs about 4 g, an adult rat could be given three pellets per day to provide about 12 g of food. However, the caloric density of the diet and the ease with which the food can be ingested are also important factors in determining the amount of time and food consumption necessary to maintain an acceptable body weight. For example, rats can ingest liquid diets quickly as compared to standard rodent pellets.

Rats fed on food restriction schedules initially lose weight but eventually undergo behavioral and metabolic changes that lead to more efficient use of nutrients (37, 57–59). Rats maintained on the same daily allotment of calories maintain equivalent weights if food is provided as one or two meals per day (60). However, several studies found that mice may die if maintained on restricted access to food, especially if they are housed alone or are fed during the light phase of the circadian cycle (61–64). Strain, arousal patterns, and effects of housing conditions on thermoregulation and energy utilization may contribute to these findings (59). For example, increased housing densities are associated with decreased food intakes and body weights in mice (25). In addition, guinea pigs may adapt poorly to a food restriction schedule (65), and they may not adjust their food consumption to adequately compensate for caloric dilution of the diet (66). However, with appropriate adaptation and training, both mice and guinea pigs can learn to perform operant responses to obtain food (66–68). Such findings highlight the importance of gradually adapting animals to a food restriction schedule by allowing them adequate time to recognize and anticipate the limited availability of food.

The circadian timing of food presentation can influence the design of food restriction schedules. Rats normally consume 70 to 85% of their daily intake during the dark phase of the circadian cycle (69–71). Food intake is modulated by circadian signals even in hungry animals, and both the satiating capacity of ingested food and the depriving capacity of inter-meal intervals show cyclic circadian variation in magnitude. Hungry rats generally eat more if food is available during the dark rather than the light phase of the circadian cycle (72, 73). Rats on food restriction protocols ideally should receive daily allotments of food near or during the dark phase of the circadian cycle.

In summary, after 24 h of food deprivation or a moderate degree of caloric restriction, animals will be hungry, but additional food may not be necessary for maintenance of homeostasis or for health. Food restriction resulting in a decrease to 80 to 85% of baseline body weights is generally sufficient for behavioral training, and caloric restriction limiting body weight to that degree promotes longevity and long-term good health. The psychological impact of chronic food restriction is more difficult to assess. Food restriction schedules may never permit the animal to eat to the point of satiation, and therefore food-restricted animals may experience some degree of chronic hunger. However, as with water intake, animals easily learn to modify their ingestion patterns to accommodate scheduled access to food. Studies with humans suggest that food restriction causes preoccupation with food and eating, but not necessarily stress. Nonetheless, careful monitoring is crucial to ensure that food-restricted animals maintain adequate intake and do not become emaciated.

Food or Water Restriction as a Motivational Tool

Three types of research protocols commonly employ food or water restriction: 1) studies of the homeostatic regulation of energy metabolism and fluid balance, 2) studies of the specific

motivated behaviors of hunger and thirst, and 3) studies that use deprivation to motivate animals to learn novel behaviors or to perform learned behaviors. Studies in the first two categories use restriction to create the drive or physiologic state that will be studied. In contrast, studies in the third category use restriction to motivate the animal to learn or perform a task that is totally unrelated to the deprivation. For example, water restriction may be used to motivate an animal to press a bar repetitively or to perform a discrimination task in studies whose goal is to measure neuronal activity during performance of the learned task. This dichotomy between the method and the goal often raises concerns about whether the restriction is necessary or can be adequately justified. The challenge of using restriction as a motivational tool for behavioral training is to achieve a balance between the severity of the imposed restriction schedule and the need to motivate learning or performance. During initial phases of training, the animal should be adapted to the restriction schedule gradually, enabling the animal to learn that food or water availability is becoming limited and context-dependent. During some phases of training, a stringent restriction schedule may be necessary to generate adequate motivation for learning or performing a difficult task, but the stringency may be reduced as the animal becomes experienced and learns the task.

An important question is whether palatable rewards can motivate behavioral performance that is equivalent to performance obtained by using restriction schedules. Access to palatable fluid can motivate an animal to perform without deprivation in some situations (74–76), particularly if the animal knows the task and if the task is relatively simple. However, satiated animals given a palatable reward may take longer to learn a task than do deprived animals, or they may perform the task poorly or not at all (16, 77–79). Furthermore, many studies require animals to perform very complex or specific tasks in a very accurate and repetitive manner for perhaps hundreds of trials in a daily session. The consistent behavioral performance required by most experimental studies cannot practically be achieved without some level of restriction-related motivation. Consider, for example, that a person was placed in a room with a water fountain that occasionally spewed water from its drinking spout. When the person first entered and explored the room, he might investigate the fountain and perhaps even take a drink, but he would probably have little sustained interest in the fountain unless he was thirsty. In that case, he would probably immediately take a drink. If he received only a tiny sip and then the water stopped, he might try to figure out how to get the fountain to deliver water more consistently. A person might be willing to exert a limited amount of effort to obtain a few sips of a tasty beverage, but he or she probably would not be motivated to work hard or consistently for that reward. This situation is analogous to that of a water-restricted animal during training to perform an operant learning task. In general, the degree of deprivation that is necessary to generate sustained interest in accomplishing the task is related to how difficult the task is to learn and perform. As with people, animals require motivation to learn or repeatedly perform challenging repetitive tasks. Restricting food or water access is necessary to achieve reliable performance under such circumstances.

Studies that require the use of restriction schedules generally employ one of two basic designs. Animals either are permitted to work for reinforcement until they become satiated and stop spontaneously, or they are permitted to work for a limited period of time each day. In the first situation, access to food or water is typically available only when the animal has the opportunity to perform the task, and supplemental food or water is not provided routinely. In the second case, the animal may be given either a fixed amount of food or water as a supplement after the work session has ended, or the animal may receive a

supplemental fixed time period during which additional consumption is permitted. An important part of the training under either scenario is teaching the animal that access to the reward is context dependent: either work is required for all access, or access is available for only a limited period of time each day. Returning to the drinking fountain analogy, if a person had learned from experience that the fountain was the only available source of water and that there would be no access to it again until the next day, he or she would probably be more inclined to exert the effort necessary to drink when the opportunity arose, even if operating the fountain was very difficult. Without the constraint of limited availability, the person might simply prefer to remain thirsty until water became more easily available. Animals generally respond in this way as well. In water restriction experiments, animals that are permitted to work and consume water until they spontaneously stop working have presumably become satiated and rehydrated when they stop. Providing supplemental water in this case may significantly interfere with either their training or their performance, because they may learn to anticipate the supplemental water and therefore will not work as hard. Rather than routinely providing supplemental water in such cases, daily intake associated with behavioral performance should instead be closely monitored to ensure that consumption is adequate to meet minimal daily needs. Periodic monitoring of body weight can aid in assessing adequate intake on a long-term basis. In experiments that do not permit the animals to work to satiation, supplemental access to water may be necessary.

The degree of restriction that is necessary to elicit adequate task performance depends on the nature of the task and should be determined for each specific behavioral model. For example, in one experiment involving a very simple bar-pressing task, rats that were deprived of water for 21 h per day quickly learned to perform this task for water, but rats that were deprived for only 7 or 14 h did not perform the task and instead simply managed on less water for a few hours (16). When the less-restricted animals were transferred to the more stringent 21-h restriction schedule, they quickly learned and performed the task, whereas rats that had learned the task showed diminished performance when transferred from a more restrictive to a less restrictive schedule (16). Similar results were obtained from rats maintained on a food restriction schedule (79). These findings indicate that significant restriction may be continually necessary to obtain acceptable performance. Conversely, an extremely stringent restriction schedule may not be necessary. In another study, rats that were maintained on various daily water rations could press a bar to obtain their allotment each day (77). Rats allotted 100% of their ad libitum intake did not perform the task at all, and rats allotted 75% of their normal intake performed very poorly (77). Again, mild restriction was inadequate for obtaining reliable task performance. However, bar-press rates were similar when water restriction was more stringent (56%, 42%, or 32% of the average daily ad libitum intake), indicating that the most severe restriction was not essential for achieving maximal performance in this task. An important consideration in evaluating these studies is recognizing that the behavioral requirements in all of the studies cited here were relatively modest. Requiring animals to perform more complicated tasks, including bar pressing under more demanding reinforcement schedules, may require more stringent restriction protocols. Therefore, a deprivation period used in one study may not provide adequate motivation for learning or performing other more demanding tasks.

The degree of restriction that is necessary may vary among individual animals. Individual animals and persons can generally be viewed as either physiologic regulators or behavioral regulators of fluid homeostasis. Physiologic regulators generally respond to moderate levels of dehydration by conserving water and excreting concentrated urine, whereas behavioral regula-

tors respond by drinking more water and excreting dilute urine (80). For example, normal dogs that are injected intravenously with concentrated saline but have no opportunity to drink excrete a concentrated urine and eliminate the salt load within 3 h (80). However, if water is available, dogs that are behavioral regulators will drink substantial amounts, apparently to alleviate thirst, and will eventually excrete the salt load in a relatively dilute urine. In contrast, dogs that are physiologic regulators will drink relatively little but instead will excrete more concentrated urine. Individual animals that are physiologic regulators can be problematic subjects for some motivation models, because they maintain hydrational homeostasis during limited availability of water by physiologic means rather than by modifying their behavior. Such animals may not be highly motivated to work for fluid reinforcement.

In summary, the degree of food or water restriction that is necessary to promote consistent behavioral performance is influenced by the difficulty of the required task, the experience of the animal, and individual coping styles. A crucial aspect of behavioral training is teaching the animal that food or water availability is limited. Food or water restriction motivates the animal to perform the behavioral task and to consume in anticipation of an expected absence of available food or water. Animals must be carefully monitored to ensure that they consume sufficient food and water each day to meet minimal homeostatic needs. Close monitoring is particularly important when the animal is first learning a restriction schedule or a behavioral task, or when the animal is required to perform a very difficult or complicated task. If task performance is not adequately supporting minimal intakes, the experimenter should reevaluate and perhaps simplify the training strategy to facilitate the animal's ability to learn and master the task. In determining whether supplemental access to fluid or nutrients should be provided, consideration must also be given to whether the animal is normally permitted to work to satiation. In general, however, animals easily learn to live under scheduled access to food or water, and their ingestion patterns can be modified as they learn to consume large proportions of their daily requirements in relatively short periods (81).

Overall Summary

As posited by Collier (29), an animal satisfying its fluid and nutritional requirements does not simply respond to its immediate internal state as hunger or thirst develop, but the animal instead learns to anticipate its nutritive requirements so that they can be met within the constraints imposed by the environment and ecological niche. Unless deficits are severe or chronic, the momentary metabolic or hydrational state of an animal has relatively little influence on its overall or long-term well-being. What is crucial is whether the animal learns to modify its patterns of ingestion to adjust to the overall availability of feeding and drinking opportunities across days. This situation is faced both by animals living in natural environments and by animals in laboratory restriction models. Although food and water may not be readily available when animals first become hungry or thirsty, they can easily manage this type of homeostatic challenge physiologically and behaviorally. If the imposed challenge is within reasonable limits, it should not cause normal animals to experience excessive or unnatural hardship or stress.

Acknowledgments

The authors thank Drs. Ed Stricker, Nancy Ator, Jerry Olson, Roger Wilson, Elizabeth Ford, and Julia Cay Jones for review of preliminary versions of this manuscript. This work was supported in part by grant CA-21765 from the National Cancer Institute and by the American Lebanese Syrian Associated Charities (ALSAC).

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