Fasting Biochemistry of Representative Spontaneous and Facultative Hibernators: The White-Tailed Prairie Dog and the Black-Tailed Prairie Dog

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Abstract

The white-tailed prairie dog is a spontaneous hibernator that enters an anorexic state followed by torpor in early fall. The black-tailed prairie dog is a facultative hibernator that enters torpor only when deprived of food and water in the winter. The physiological state of hibernation is similar to a Phase II euthermic fast characterized by elevated fat catabolism, increased blood ketone bodies, and conservation of protein tissues. It was hypothesized that these spontaneous and facultative hibernators use fat and protein differently during a fast prior to the hibernation season. Weekly blood and urine samples were taken from both species during a 5-wk period of food and water deprivation. The black-tailed prairie dog lost mass at a greater rate and had a larger daily urine volume and urea, ammonia, and potassium excretion, as well as a higher plasma urea/creatinine ratio, all of which define a greater rate of protein catabolism for this species than for the white-tailed prairie dog. The black-tailed prairie dog, therefore, does not conserve protein to the same extent as the white-tailed prairie dog during a Phase II fast typical of hibernation starvation. Ketone bodies do not appear to regulate protein catabolism directly. But the greater protein catabolism by the black-tailed prairie dog may be related to pH and water balance requirements that are circumvented in the white-tailed prairie dog by engaging in spontaneous torpor. Both species evolved from populations of ancestral prairie dogs that have retained the ability to hibernate spontaneously. It is hypothesized that the black-tailed prairie dogs may not have maintained the capacity for a deep Phase II, protein-conserving state typical of hibernation starvation but keep an active profile throughout winter, relying to a greater extent on protein catabolism in response to selective pressures of greater predation, higher food abundance, and perhaps a need to preserve fat stores for reproduction.

Introduction

Hibernation is one solution to the problem of seasonal food and water shortage. Most temperate mammals, however, maintain their body temperature within a very narrow range and have not adopted this strategy. As pointed out by French (1988, 1989), it may be important for mammals to maintain a normal body temperature in order to provide a greater opportunity for such activities as social behavior and reproduction. In this regard, two groups of mammals with the general capacity to hibernate have been identified: spontaneous and facultative hibernators (Folk 1974, pp. 280–292). Spontaneous hibernators enter torpor in response to a persistent circannual rhythm (Davis 1976; Mrosovsky 1978) or photoperiod cues (Pengelley and Asmundson 1972) even when they have available food and water (Mrosovsky and Powley 1977). However, facultative hibernators remain at a normal body temperature unless they receive an additional exogenous stressor such as food or water shortage to initiate torpor. Two closely related species which demonstrate these different adaptive strategies for winter survival are the white-tailed prairie dog (*Cynomys leucurus*) and the black-tailed prairie dog (*Cynomys ludovicianus*). While the white-tailed prairie dog is more intermontane in distribution (Hall and Kelson 1959), both species are sympatric or live in similar habitats in many parts of their range (Long 1965). The black-tailed prairie dog can be found above ground throughout the winter (King 1955; Koford 1958; Smith 1958; Bakko 1977; Bakko, Porter, and Wunder 1988) and, as may be expected, it is the more social of the two species (Tileston and Lechleitner 1966). On the other hand, the white-tailed prairie dog is less social and retreats below ground between July and early August for the winter season.

In laboratory studies, Harlow and Menkens (1986) demonstrated the white-tailed prairie dog to be a spontaneous seasonal hibernator while the black-tailed prairie dog required extensive food and water deprivation at 6°C before it entered torpor. It may be that conditions under which the black-tailed prairie dog evolved were not selective for rigid circannual bouts of spontaneous hibernation, and, during the past 3 million yr, the black-tailed prairie dog adapted alternate physiological mechanisms for winter survival, that is, the use of deep torpor only in times of extreme cold and food shortage. However, the physiological processes that account for the differences in hibernation ability by these two species are not known. Several possibilities have been proposed, which include differences in (1) renal structure and function, (2) body fat content, and (3) fasting capacity.

With reference to these three possibilities, in the first case, Bakko (1977) proposed that the black-tailed prairie dog has evolved renal structures that

allow it to highly concentrate urine, thereby reducing the need for hibernation to avoid winter dehydration. However, in another study Harlow and Braun (1995) could not identify major differences in the renal morphology of these two species and concluded that the higher urine osmolality observed in the black-tailed prairie dog (Bakko 1977) may be a result of elevated urinary urea concentrations associated with higher protein catabolism while fasting. In the second case, there does not appear to be a significant difference in body fat content between these two species in early fall (H. J. Harlow, unpublished data). With respect to the third possibility, it does appear that these two species may exhibit differences in fasting physiology.

Hibernation has often been referred to as a biochemical state comparable to prolonged fasting. Hibernating squirrels have very little stored glycogen, but they require glucose as a substrate for cellular respiration of certain tissues and utilize ketone bodies as an inhibitor of gluconeogenesis (Krilowicz 1985). When provided *ad lib.* food and water in late fall and held at 6°C with low-lux lighting, the white-tailed prairie dog goes into spontaneous anorexia just before hibernation while the black-tailed prairie dog continues to eat and stay euthermic (H. J. Harlow, unpublished data). The question posed in this study is whether the white-tailed prairie dog and black-tailed prairie dog have different mechanisms of using fat and protein while fasting that influence their propensity to hibernate.

During prolonged food deprivation, animals progress through two phases, each characterized by a distinct profile of substrate utilization (Cahill 1976; Robin et al. 1988). Early in a fast (Phase I), carbohydrates and protein are catabolized, denoted by high blood glucose and urea/creatinine (U/C) ratio but low ketone body (acetone, acetoacetic acid, and β -hydroxybutyric acid) concentrations (Cahill 1976). Within several days, a fasting animal starts to enter Phase II with elevated plasma ketone bodies (Robinson and Williamson 1980) but a depressed glucose concentration and U/C ratio indicative of reduced protein catabolism. Urinary potassium, urea, and ammonia generally decrease during this stage, which also identifies a period of protein conservation. As an animal enters Phase II starvation, ketone bodies (primarily β -hydroxybutyric acid) are believed to regulate gluconeogenesis (Nosadini et al. 1981) and cellular glucose uptake (Robinson and Williamson 1980) as well as to balance the urinary excretion of ammonia (Sigler 1975; Hannaford et al. 1982). Ketone bodies are therefore thought to be important regulators of protein catabolism during a Phase II fast (Nosadini et al. 1981) and during hibernation starvation (Krilowicz 1985).

A recent study by Thompson, Agar, and Bintz (1993) noted that black-tailed prairie dogs appear to rely primarily on tissue protein during periods of inclement weather and that these animals have a relatively slow rate of

lipid turnover. The objectives of the present study are to determine the relative extent of a Phase II fast by these two species and distinguish the role of ketone bodies in protein conservation by monitoring body mass and blood and urine metabolites. Facultative hibernation by the black-tailed prairie dog may be a derived state and limited by greater reliance on protein catabolism, while spontaneous hibernation by white-tailed prairie dogs may be more closely related to the mountain ancestral stock and characterized by a heavier dependence on fat catabolism and greater protein conservation.

Material and Methods

Eight white-tailed and eight black-tailed prairie dogs were livetrapped in southeastern Wyoming and northern Colorado during July. The habitat from which the white-tailed prairie dogs were removed (41° N, 105° W; elevation 2,215 m) is dominated by two species of grass (western wheatgrass [*Agropyron smithii*] and blue grama [*Bouteloua gracilis*]), one species of forb (snakeweed [*Gutierrezia* sp.]), and a subshrub (fringed sage [*Artemisia frigida*]) (G. E. Menkens and S. H. Anderson, unpublished data). Mean minimum temperature in the region occurs in January (−6.2°C), with an average total precipitation between 25 and 30 cm, mostly occurring between March and October. The habitat from which the black-tailed prairie dogs were trapped (40° N, 106° W; elevation 1,525 m) was characterized by very short vegetation (as a result of clipping by the prairie dogs), and the area was dominated by two species of perennial grasses: blue grama and buffalo grass (*Buchloe dactyloides*) (Klatt and Hein 1978). Mean minimum temperature in the region occurs in January (−2.8°C), with an average total precipitation between 35 and 40 cm, mostly occurring between March and August (Klatt and Hein 1978).

The fasting study to be described was based on conditions similar to those experienced by animals during their normal life cycle. Prairie dogs undergo natural periods of prolonged food and water deprivation, which can extend from 4 to 6 wk at normal body temperatures and to over 4 mo when the animals are engaged in torpor. Prairie dogs in this study were maintained in individual metal metabolic cages 25 cm × 20 cm × 17 cm housed in a room exposed to natural photoperiod and ambient temperature. All animals were maintained on ad lib. food (Purina Lab Chow) and water until the first part of August, when food and water were removed. Urine samples were collected over a 24-h period at the end of each week of a fast for 5 wk. The health and vigor of these animals was assessed daily by observing their activity, responsiveness to handling, body mass, and coat color. In order to

obtain a fresh sample of urine, funnels beneath each cage were fitted with two small wires. When the wires became bridged by a drop of urine, a signal was transmitted remotely to alert a technician to remove the fresh sample. All urine was collected in a beaker with mineral oil to reduce evaporation, immediately measured for volume, and frozen at -20°C . Once a fresh sample was obtained, the remaining 24-h sample was simply combined for volume measurement and discarded. In the morning (0800–1000 hours) at the termination of a 24-h collection day, each animal was anesthetized with Ketamine hydrochloride (0.02 mg/g body mass), weighed, and a 1-mL blood sample obtained (in lithium Heparin) by cardiac puncture. Blood samples were placed on ice and centrifuged for harvesting plasma, which was then frozen at -20°C .

An aliquot of the urine samples was measured for total osmolality on a Wescor model 5100C vapor pressure osmometer. Potassium was also measured on this portion of urine with a Perkin Elmer model 51a flame photometer. Additional aliquots of urine and of the plasma samples were analyzed for urea according to the diacetyl method using thiosemicarbazide as described by Harlow and Seal (1981), creatinine was measured colorimetrically with reagents prepared by Sigma Chemical Company (no. 555-A), and β -hydroxybutyric acid was measured according to the colorimetric method of Wildenhoff (1970). Ammonia was determined on an aliquot of the urine samples by the Urease/Berthelot reaction. Glucose was determined on portions of plasma samples according to the method described by Harlow and Seal (1981).

A one-way ANOVA with repeated-measures design was employed with a Scheffe post hoc test to identify significant changes during the fast for the various parameters monitored. A paired *t*-test was used to detect differences between species at each week during the fast. Significance is defined at the $P < 0.05$ level.

Results

At the beginning of the study, white-tailed and black-tailed prairie dogs were of similar body masses; however, the rate of mass loss during fasting was greater for the black-tailed prairie dogs. By the end of the first week of fasting, the black-tailed prairie dogs had a significantly lower body mass than white-tailed prairie dogs (fig. 1).

Urine osmolality of white-tailed prairie dogs decreased throughout the fast, but it increased for the black-tailed prairie dogs such that their concentration was significantly higher than that of the white-tailed prairie dogs

Fig. 1. Body mass (g) of eight white-tailed prairie dogs and eight black-tailed prairie dogs prior to and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 standard errors of the mean (SEM).

after 5 wk of fasting (table 1). Urine potassium significantly decreased for both species, but concentrations for black-tailed prairie dog urine were significantly higher than for white-tailed prairie dog urine at the end of the fast (table 1). Urine urea decreased during the fast for white-tailed prairie dogs but increased for black-tailed prairie dogs so that it was significantly higher than the concentration for white-tailed prairie dogs after 5 wk of fasting (table 1). Urinary ammonia increased in both species and was not significantly different between species at the end of the fast (table 1). There was no significant alteration of urinary β -hydroxybutyric acid by the end of the fast in either species; however, concentrations for the white-tailed prairie dogs were higher than black-tailed prairie dogs throughout the study (table 1). Plasma levels of β -hydroxybutyric acid decreased in white-tailed prairie dogs but increased in black-tailed prairie dogs, with plasma levels significantly higher for black-tailed prairie dogs at the end of 5 wk fasting (table 1). Plasma glucose levels declined during the study but were not

TABLE 1
Mean plasma β -hydroxybutyric acid and glucose concentrations and urine osmolality, potassium, urea, β -hydroxybutyric acid, and ammonia concentrations for eight white-tailed prairie dogs and eight black-tailed prairie dogs before and after 5 wk of food and water deprivation

Sample	White-Tailed Prairie Dog		Black-Tailed Prairie Dog	
	Prefast	5-wk Fast	Prefast	5-wk Fast
Plasma:				
β -Hydroxybutyric acid (μ molar) . . .	231.0 (24.0)	158.5 (22.4)	288.5 (49.5)	469.0 (61.0)
Glucose (mmolar)	9.8 (.8)	7.4 (.5)	11.5 (.7)	6.4 (.4)
Urine:				
Osmolality (mosm/L)	2,382.0 (224.0)	1,710.0 (80.0)	1,326.0 (216.0)	2,598.0 (97.0)
Potassium (mEq/L)	435.0 (42.0)	71.0 (20.0)	214.0 (33.0)	154.0 (26.0)
Urea (mmolar)	1,908.0 (173.0)	1,504.0 (39.0)	1,138.0 (164.0)	2,460.0 (110.0)
β -Hydroxybutyric acid (μ molar) . . .	5,982.0 (810.0)	6,420.0 (620.0)	3,941.0 (781.0)	4,188.0 (189.0)
Ammonia (mmolar)	48.3 (2.8)	303.6 (82.0)	71.2 (5.4)	234.0 (24.0)

Note. Values in parentheses are ± 2 SEM.

significantly different between species at the end of the fast (table 1). These data are in agreement with many of the values reported on fasting black-tailed prairie dogs by Pfeiffer, Reinking, and Hamilton (1979).

Urine production, measured as mL/d, was significantly reduced by the first week of the fast. Black-tailed prairie dogs had a significantly higher daily urine volume than white-tailed prairie dogs throughout the fast (fig. 2). Plasma U/C ratio was significantly elevated from prefast values in both species by the end of the fast. The plasma U/C ratio was greater for black-tailed prairie dogs during weeks 3, 4, and 5 of the fast (fig. 3). Daily urinary urea, ammonia, and β -hydroxybutyric acid excretion was significantly reduced in both species of prairie dogs at the end of the first week of fasting but was significantly higher for the black-tailed prairie dog throughout the fast (figs. 4–6). Urinary potassium excretion was also significantly reduced in both species by the end of the first week, but black-tailed prairie dogs

Fig. 2. Daily urine volume (mL) for eight white-tailed prairie dogs and eight black-tailed prairie dogs prior to and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 SEM.

Fig. 3. Plasma U/C ratios for eight white-tailed prairie dogs and eight black-tailed prairie dogs before and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 SEM.

had a higher daily excretion of potassium than the white-tailed prairie dogs during the entire fast except for week 3 (fig. 7).

Discussion

Just what is it that allows some animals to engage in circannual, spontaneous torpor bouts while other animals are facultative and use torpor only under severe climatic and energy constraints? The white-tailed prairie dog and black-tailed prairie dog provide a model to investigate this question owing to their phylogenetic history, present distribution, and differences in hibernation ability. *Cynomys* is most closely allied to the genus *Spermophilus* and became separated from this line in the southern Rocky Mountains during the Pliocene. The diploid number of the Gunnison's prairie dog, *Cynomys gunnisoni*, is lower than that of other *Cynomys* species; it more closely resembles *Spermophilus* and, therefore, may be the ancestral stock of the

Fig. 4. Daily urinary urea excretion (mmol/d) for eight white-tailed prairie dogs and eight black-tailed prairie dogs before and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 SEM.

white-tailed and black-tailed prairie dogs (Pizzimenti 1975). It is thought that marginal populations related to *C. gunnisoni* underwent chromosomal remodeling characteristic of the other species of *Cynomys* and spread westward into the Great Basin to become the white-tailed prairie dog lineage and eastward into the Great Plains to become the black-tailed prairie dog lineage. Changes in climatic conditions and uplift of plateaus separated these populations. Selection pressures east of the divide were probably different owing to a more open, xeric environment that lacked refugia (Pizzimenti 1975). It is possible that biotic and abiotic conditions during the past 3 million yr in the Great Plains did not select to maintain spontaneous hibernation for the black-tailed prairie dog as a winter survival strategy, which was more characteristic of Gunnison's and white-tailed prairie dogs.

Various environmental constraints resulting in physiological responses can be proposed to account for observed differences in hibernation ability for these two closely related species (Harlow and Menkens 1986). Some

Fig. 5. Daily urinary ammonia excretion (mmol/d) for eight white-tailed prairie dogs and eight black-tailed prairie dogs before and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 SEM.

researchers have concluded that the reason black-tailed prairie dogs can be facultative hibernators is that they have kidneys better designed to deal with potential winter dehydration (Bakko 1977). These conclusions have been based on measurements of a greater urine concentration and on a larger relative medullary thickness (an indicator of the length of the renal papilla) for the black-tailed prairie dog. However, our measurements of the relative medullary thickness, as well as other morphological characteristics of the kidney from these two species, showed no distinct differences (Harlow and Braun, 1995). In addition, both species produced identical urine concentrations when salt loaded, but when they were food and water deprived, the black-tailed prairie dog had higher urea and osmotic levels, which suggests that this species may rely more on protein reserves than does the white-tailed prairie dog (Harlow and Braun 1995). These data are supported by Thompson et al. (1993), who documented that black-tailed prairie dogs killed in the field (one group before and one group after 20 d of natural

Fig. 6. Daily urinary β -hydroxybutyric acid excretion ($\mu\text{mol/d}$) for eight white-tailed prairie dogs and eight black-tailed prairie dogs before and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 SEM.

fasting), demonstrated a large drop in protein mass. These authors concluded that protein, not fat, was the major fuel source for black-tailed prairie dogs forced to fast under natural conditions. Similarly, the present study demonstrates a high metabolic dependence on protein by the black-tailed prairie dog.

The extent of protein conservation during Phase II of a fast is reflected in the amount of protein catabolized and can be monitored by alterations in body mass in concert with blood and urine chemistry profiles. Both species entered a Phase II fast by the first week of food and water deprivation. However, data from this study demonstrate that the black-tailed prairie dog did not enter a protein-conserving Phase II fast to the same extent as the white-tailed prairie dog. There are several parameters that support this conclusion. First, even though both species demonstrated similar activity profiles and body temperatures during the fast and are reported to have similar metabolic rates (Harlow 1987), the black-tailed prairie dog had a greater

Fig. 7. Daily urinary potassium excretion (mEq/d) for eight white-tailed prairie dogs and eight black-tailed prairie dogs before and at weekly intervals during 5 wk of food and water deprivation. Circles, white-tailed prairie dogs; squares, black-tailed prairie dogs. Vertical bars represent ± 2 SEM.

rate of body mass loss during the fast. This greater mass loss by the black-tailed prairie dog may have resulted from the preferential catabolism of protein, which is lower in mass-specific energy and higher in bound water than fat (Bintz et al. 1979). As a result, black-tailed prairie dogs would require a greater mass of protein to provide a comparable energy yield. Second, the elevated water and nitrogen release from a higher protein catabolism in the black-tailed prairie dog would account for the elevated plasma ammonia and urea levels as well as higher urine volume and daily excretion of urea and ammonia in these animals. This line of evidence is supported by higher plasma U/C ratios in the black-tailed prairie dog. Third, black-tailed prairie dogs excreted a greater amount of potassium in their urine each day. Intracellular potassium concentrations are approximately 130 mEq/kg of muscle tissue, which, when catabolized, results in a concomitant elevated urinary potassium (Graham 1970). Daily urine potassium excretion suggests a greater reduction in muscle protein catabolism by white-

tailed prairie dogs during all but 1 wk of the study. The proportion of fat and protein catabolized at the end of the fast was determined from daily excretion of urinary nitrogen (millimoles of ammonia and urea) and caloric equivalence of total metabolism calculated from oxygen consumption. Protein breakdown was estimated from the relationship that 1 g of urinary nitrogen is equivalent to 6.25 g of degraded protein (Runcie and Hilditch 1974). The energy equivalence of daily protein use (23 kJ/g) was subtracted from the energy equivalence of daily oxygen consumption (19.5 kJ/L O₂) (Withers 1992, p. 86). Assuming that glycogen stores are depleted within the first few days of the fast (Cahill and Owen 1967), this difference represents energy derived from fat. Fat-to-protein ratios of 4:1 and 40:1 were calculated for black-tailed prairie dogs and white-tailed prairie dogs, respectively, at the end of the fast.

The question that now arises is what determines this ratio of fat to protein catabolized. Four factors potentially affect this process: (1) the initial body fat content, (2) the need for protein catabolism to provide three- and four-carbon citric acid cycle intermediates necessary for fat catabolism, (3) the role of fat, via ketone bodies, in regulating protein catabolism, and (4) the interrelationship of fat and protein catabolism for water to maintain body hydration.

In regard to the first point, fat content of animals removed from the field in August showed no significant difference between species (H. J. Harlow, unpublished data). In considering the second point, Yacoe (1983) speculates that in order to catabolize a specific amount of fat, there must be a constant proportion of protein breakdown to produce three- and four-carbon intermediates to the citric acid cycle to sustain the fat catabolism (Lee and Davis 1979). However, in another study comparing the fasting biochemistry of the pine marten and the black-tailed prairie dog (Harlow and Buskirk 1991), the ratio of fat to protein was disproportionate, which does not support Yacoe's (1983) hypothesis.

In regard to the third possibility, the fat-to-protein ratio may be regulated by the amount of ketone bodies. It is thought that β -hydroxybutyric acid is instrumental in sparing protein during Phase II of a fast (Williamson and Whitelaw 1978) by depressing glucose uptake in cells as well as inhibiting the production of alanine required for gluconeogenic activity (Odessey, Khairallah, and Goldberg 1974). With this scenario, it would be expected that the species with the highest fat and lowest protein catabolism would have the highest plasma β -hydroxybutyric acid levels. However, it was the black-tailed prairie dog with its elevated protein catabolism that had the highest plasma β -hydroxybutyric acid concentrations. This suggests that ketone bodies (specifically, β -hydroxybutyric acid) are not acting to suppress

protein catabolism in these species. This is consistent with previous findings on the prairie dog and pine marten (Harlow and Buskirk 1991).

On the other hand, it has been claimed that ammonia and ketone bodies are excreted in proportional amounts in order to maintain the acid-base status of the individual (Sigler 1975; Halperin, Goldstein, and Stinebaugh 1982). It is possible that a higher protein catabolism in the black-tailed prairie dog produced an elevated urinary ammonia nitrogen excretion accompanied by a concomitantly high ketone body excretion. Therefore, while the elevated ketone body level in the black-tailed prairie dog may not have had a regulatory effect on protein catabolism via glucose uptake or inhibition of gluconeogenesis, it may have had an influence on protein breakdown via acid-base balance. The white-tailed prairie dog, with its higher fat-to-protein ratio had lower plasma ketone body concentrations, as well as lower urinary ammonia and ketone body excretion.

A mechanism acting independently or in concert with ketone body regulation of protein catabolism may be operating in response to the need for water and maintenance of tissue hydration during fasting. Lipid catabolism is a major source of metabolic water. However, as pointed out by Bintz and associates (Bintz and Riedesel 1967; Bintz et al. 1979; Bintz and Mackin 1980), fat is stored in a dehydrated form, and there is a large insensible respiratory water loss causing a negative water balance from catabolism of adipose tissue alone (Chew 1965). But free water represents about 75% of the mass of muscle tissue, in contrast to its relative absence in stored lipid (Allen 1976). Therefore, during fasting and water deprivation, catabolism of tissues with high protein can allow a positive water balance (Bintz et al. 1979). However, protein contains less energy than the same amount of fat. Therefore, an animal may derive the greatest part of its calories from oxidation of fat during fasting and catabolize just sufficient proteinaceous tissue to maintain water balance. Bintz et al. (1979) has advanced the hypothesis that the degree of tissue hydration may serve as a stimulus for selective tissue catabolism during a fast. Under these conditions, the white-tailed and black-tailed prairie dogs could adjust their water balance by catabolizing a specific ratio of fat to protein. The ratio of 4:1 fat-to-protein catabolism for the black-tailed prairie dog approximates that reported by Bintz et al. (1979) to maintain water balance in ground squirrels and, therefore, may also be sufficient to maintain hydration during fasting for this species as well. The white-tailed prairie dog, on the other hand, catabolized far less protein and may have difficulty staying in water balance during fasting.

Mean urine urea and osmolality was higher for the white-tailed prairie dog during the prefast period. This was primarily a result of three individuals with unusually high urinary urea and consequent urine osmolality. These

individuals were more in line with the rest of the group during subsequent sampling periods. In spite of the relatively high prefast urine urea value for white-tailed prairie dogs, their lower mean urine volume resulted in a daily excretion similar to that of the black-tailed prairie dog during the prefast period and contributed to a lower daily urea excretion during the fast. The higher urine volume for black-tailed prairie dogs during the fast is consistent with a greater need to expel urea resulting from higher protein catabolism. As previously discussed, Harlow and Braun (1995) failed to demonstrate marked differences in kidney structure between these two species. Therefore, renal concentrating capacity may not be greater for the black-tailed prairie dog, and the higher urine osmolality reported in this study as well as other studies (Bakko 1977; Harlow and Braun 1995) may represent only a greater amount of urea, ammonia, ketone bodies, and potassium excreted as a result of higher protein catabolism. While many factors influence water loss, Bintz et al. (1979) speculates that water balance may be enhanced during such a condition through reducing respiratory water loss associated with metabolic depression and living in a highly humid burrow. The white-tailed prairie dog may, therefore, rely upon torpor and continuous winter inhabitation within a plugged burrow with highly saturated air and reduced respiratory water loss. On the other hand, while the black-tailed prairie dog conserves protein during a fast, it still retains a fat-to-protein ratio that may aid in maintaining water balance until conditions become extreme. Indeed, Harlow and Menkens (1986) found that extended water deprivation during midwinter enhanced the onset of torpor in this species.

The recent article by Thompson et al. (1993) proposed that the black-tailed prairie dog has less body fat and lower rates of fat synthesis than ground squirrels. They speculated that the black-tailed prairie dog uses protein as a fuel during much of the winter and reserves fat for reproduction during March and April. While white-tailed and black-tailed prairie dogs appear to have comparable fat stores in the fall prior to hibernation (H. J. Harlow, unpublished data), the present study is in agreement with Thompson et al. (1993) in that the black-tailed prairie dog does utilize less fat during a fall fast and relies more on tissue protein. An enhanced adaptive advantage could be realized from this higher protein catabolism if the black-tailed prairie dog has labile protein stores. Many vertebrates, including birds (Jones and Ward 1976; Le Maho et al. 1981), fish (Kendall, Ward, and Bacchus 1973), and mammals (Millward and Waterlow 1978; Yacoe 1983; Torbit et al. 1985) use protein as an energy source and store protein seasonally for this purpose. These protein reserves appear to be in specific compartments, including the skin and viscera (Millward and Waterlow 1978; Torbit et al. 1985) as well as blood albumins (Garcia-Rodrigues et al. 1987). In

addition, when skeletal muscle is catabolized for energy, impacts on locomotor capacity may be minimized by using specific muscle groups (Le Maho et al. 1981) and using labile proteins stored in the sarcoplasm between the myofibrils, without affecting the sarcotubular system (Kendall et al. 1973).

The black-tailed prairie dog shows a greater divergence than the white-tailed prairie dog in hibernation strategy from the ancestral Gunnison's prairie dog (Pizzimenti 1975). Selective pressures east of the continental divide during the past 3 million yr probably resulted in physiological specializations for the black-tailed prairie dog to conserve fat rather than protein during fasting, which may reduce their propensity to hibernate. Two potential explanations can be advanced for differential fasting capacity associated with facultative hibernation by the black-tailed prairie dog. The first is that the lengthened growing season and increased food availability of the lower elevations of the Great Plains may have reduced the need for hibernation by the black-tailed prairie dog. Second, it has been proposed that the lack of refugia (which are more typical of the mountain areas) reduced the amount of protection from predation (Pizzimenti 1975). Powell (1982) pointed out that the black-tailed prairie dog and its major predator, the black-footed ferret (*Mustela nigripes*) evolved together on the Great Plains. The black-tailed prairie dog may have limited its hibernation events to times of extreme cold or food and water scarcity in order to reduce predation vulnerability associated with torpor and evolved a social structure conducive to predator avoidance. Both conditions may have resulted in a reduced dependence on hibernation. Black-tailed prairie dogs, therefore, may augment fat stores with protein stores in order to maintain pH and water balance during periods of food and water scarcity, thereby reducing their need for spontaneous hibernation, and conserve fat for reproduction. The data from this study support the premise of Srere, Wang, and Martin (1992) that the ability to hibernate is linked to a reprogramming of existing biochemical capabilities rather than the creation or loss of hibernation-specific genes.

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