Water Availability Affects Reproduction in Deer Mice

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ABSTRACT

Water restriction impaired sperm production in deer mice, a seasonally breeding mammal that encounters aperiodic droughts in its natural habitat throughout North America. Water-induced spermatogenic responses were sorted into three categories based upon epididymal sperm numbers: aspermic, oligospermic, and euspermic. Average gonadal mass was reduced after 10 wk of limited water consumption. Inter-individual variation in gonadal response to a simulated drought was similar to phenotypic differences in reproductive function in response to other environmental cues that direct annual reproductive cycles. Our findings suggest that water availability may act as a cue to suppress gametogenesis in deer mice independently from food, temperature, and day length.

INTRODUCTION

Timing of reproduction depends upon one or more cues that predict the season of the year (Sadleir, 1969). Different sensory modalities have evolved in animals to ensure that their reproductive effort coincides with the time of year most propitious for producing young. For instance, reproduction occurs during the spring and summer among most rodent species inhabiting temperate latitudes. Seasonal breeding among deer mice (Peromyscus maniculatus), one of the most common and widely distributed mammals in North America, is dependent upon a minimum of three environmental cues: photoperiod, temperature, and caloric intake (Desjardins and Lopez, 1983; Blank and Desjardins, 1985).

Among the external cues that signal reproductive effort in rodents, research has centered upon understanding how annual changes in photoperiod control autumnal reproductive regression and vernal reproductive recrudescence (Zucker et al., 1980). In addition to the absence of winter breeding, many species from the northern temperate latitudes also display a midseason hiatus in reproductive performance during the summer (Burt, 1940; Blair, 1951; Rintamaa et al., 1976; Batzli, 1977; Cornish and Bradshaw, 1978; Drickamer, 1978). The summer cessation of breeding for some rodents living in arid or xeric habitats has expanded many months so that reproductive activities are restricted to the winter (Blair, 1951; Lidicker, 1976). Few studies have addressed the environmental control of reproductive quiescence during the breeding season (Yahr and Kessler, 1975; cf. Lidicker, 1976; Nelson et al., 1983). The cited studies suggested that reduced water intake can inhibit reproduction; however, the effects of water restriction were not separated from the effects of reduced food intake (Yahr and Kessler, 1975; Lidicker, 1976; Nelson et al., 1983). Animals often limit their food intake when water availability is scarce (King, 1968), and food restriction can induce gonadal regression (Blank and Desjardins, 1985). It remained possible that food restriction, subsequent to limited water intake, inhibited breeding in the previous studies. Alternatively, reduced water intake could directly influence gonadal function.

The present study was designed to test the hypothesis that a reduction in water consumption directly affects reproductive function in rodents from mesic habitats. Deer mice reduced the size of their reproductive systems after sustained water restriction...
relative to animals provided with water ad libitum. Additional analyses revealed that three types of testicular responses were apparent after water limitation; these gonadal responses included individuals with normal, reduced, or zero sperm counts. This variation in reproductive responsiveness is analogous to phenotypic differences in the gonadal responsiveness to other external cues.

MATERIALS AND METHODS

Food (Wayne Mouse Breeding Diet; Allied Mills, Inc., Chicago, IL; 8–10% water content) and water intake (ad libitum) were monitored for 10 consecutive days for 83 individually caged adult male deer mice (*Peromyscus maniculatus nebrascensis*). All males were between 50 and 70 days of age when food and water consumption was measured. These animals were reared from F₁ descendants of 85 parental pairs trapped in South Dakota (Desjardins and Lopez, 1983). Animals were maintained in 16L:8D (lights on 0600 h, CST) throughout the study. Colony temperature was maintained at 20 ± 2°C with 50 ± 10% relative humidity. Males were weaned at 21–25 days of age, individually housed, and used after 60 days of age or about four weeks after they achieved reproductive maturity. A randomly selected subset of 18 mice received food and water ad libitum throughout the study, but were otherwise treated similarly to experimental mice. Sixty-five animals received 50% of their ad libitum water intake for 30 days. They were further restricted to 25% of their original water intake for an additional 40 days, a time span that encompasses the full duration of the cycle of the seminiferous epithelium (Desjardins and Lopez, 1983).

Animals were checked twice daily for torpor. Body mass and 24-h food intake were determined weekly. Food consumption was calculated as grams of food ingested/gram of body mass; water intake was calculated as cc of ingested water/gram of body mass. Mice were killed with a lethal injection of sodium pentobarbital. The mass of paired testes and epididymides was recorded at necropsy and spermatogenic activity was determined. Paired testes (without capsules) and epididymides were minced and transferred separately to an Eberbach blending assembly. Testes and epididymides were homogenized for 30 and 45 s, respectively, in 25 and 50 ml 0.15 M sodium chloride plus 0.05% Triton-X100 (Sigma Chemical Co., St. Louis, MO) with 0.25 M thimerosal (Sigma Chemical Co.). This technique yielded optimal numbers of elongated spermatids or spermatozoa resistant to homogenization. A sample was removed to determine the number of nuclei with a characteristic shape of mature spermatozoa or elongated spermatids under phase-contrast microscopy. Duplicate determinations were made for each homogenate.

Statistical comparisons were based upon analysis of variance. Analyses of the three reproductive phenotypes were considered post-hoc and were subjected to Scheffe's tests.

![Graph](image)

**FIG. 1.** *Top panel:* Number of sperm or elongated spermatids per paired testes for deer mice (*Peromyscus maniculatus*) receiving ad libitum or restricted water (25% of ad libitum intake). Each value is expressed as the mean ± SEM for the number of mice listed near the base of each bar. The 62 water-restricted mice exhibited a continuum of spermatogenic responses and were sorted (right of the dotted line) into three subclasses with 22 aspermic, 28 intermediate, and 12 normal animals, respectively. Homogenates of aspermic testes contained a few cells that may represent elongated spermatids, but the epididymides of these animals were devoid of spermatozoa. *Bottom panel:* Paired testes mass of deer mice receiving ad libitum or restricted water. Each value is expressed as the mean ± SEM for the number of animals listed near the base of each bar. The same criteria were applied to subdivide animals on the basis of their epididymal sperm count after water restriction.
RESULTS

The results established that limited access to water significantly ($p<0.01$) reduced testicular mass and spermatogenesis relative to control animals with unrestricted water supplies (Fig. 1). Body mass did not differ significantly between the two experimental groups ($p>0.05$) (Table 1). In addition to comparing the mean values for groups of unrestricted and restricted mice, we also assessed the spermatogenic response of each animal. All deer mice with unlimited access to water displayed sperm numbers similar to those observed in normal animals and consistent with fertility (Desjardins and Lopez, 1983). Three distinct testicular responses emerged within the water-restricted group: 1.) sperm counts were indistinguishable from those of control mice (normal; $n = 25$), 2.) animals were devoid of sperm (aspermic; $n = 17$), or 3.) sperm counts were intermediate between these classes (intermediate; $n = 22$) (Fig. 1).

Deer mice were sorted according to epididymal sperm numbers as normal (<4 standard errors of the mean [SEM] of ad libitum [$>62 \times 10^{-6}$ sperm/paired epididymides]), intermediate ($>4$ SEMs <7 SEMs of ad libitum water values [$<61$ and $>2 \times 10^{-6}$ sperm/paired epididymides]) or aspermic ($>7$ SEMs of ad libitum water values [$<2 \times 10^{-6}$ sperm/paired epididymides]). This strategy for classifying spermatogenesis is similar to that used in past studies with deer mice and the methodology has been validated for use with this species (Desjardins and Lopez, 1983; Blank and Desjardins, 1985).

There was no statistical difference in reproductive organ or body mass between water-restricted deer mice that maintained normal spermatogenesis (normal) and unrestricted animals ($p>0.05$). Testicular and epididymal mass was reduced in aspermic mice relative to mice given water ad libitum (21% and 28% reductions, respectively; $p<0.05$) (Fig. 1, Table 1).

Although not statistically significant, the average body mass of water-restricted deer mice decreased about 15% relative to their ad libitum counterparts. When all the water-restricted mice were partitioned into three classifications based upon epididymal sperm numbers, body mass was reduced 28% ($p<0.01$; aspermics), 14% ($p>0.05$; intermediates), and 6.4% ($p>0.05$; normals). After correcting for body mass, however, relative testicular mass followed the same pattern of response as absolute testicular mass (Table 1). No mice exhibited daily torpor. Further, no significant differences ($p>0.05$) were evident in relative food intake among mice with unlimited vs. restricted access to water. Finally, food and water intake was similar ($p>0.05$) among the three classes of reproductive morphs (Tables 2 and 3).

DISCUSSION

Restricted access to water significantly reduced testicular mass and spermatogenic activities relative to testicular mass and spermatogenic activities in animals with unrestricted provisions of water. Three classes of testicular response were observed among deer mice with limited water availability: 1.) some mice maintained normal sperm numbers, 2.) others became aspermic, and 3.) the remaining mice maintained sperm production at an intermediate level. Our findings have two important functional implications for seasonal reproduction: limited water availability may inhibit spermatogenesis, and phenotypic variation in responsiveness to restricted water exists within a population. These physiological changes in response to limited water intake may be specific to the reproductive system.

Importantly, water restriction did not reduce relative food intake nor did it have any apparent effect on daily torpor, two physiological responses

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body mass</th>
<th>Relative testes mass</th>
</tr>
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<tbody>
<tr>
<td>Ad libitum (n = 18)</td>
<td>23.09 ± 0.69</td>
<td>0.013 ± 0.003</td>
</tr>
<tr>
<td>Water-restricted (n = 62)</td>
<td>19.70 ± 0.82</td>
<td>0.009 ± 0.001</td>
</tr>
<tr>
<td>Aspermic (n = 22)</td>
<td>16.56 ± 0.65*</td>
<td>0.002 ± 0.001*</td>
</tr>
<tr>
<td>Intermediate (n = 28)</td>
<td>19.94 ± 0.69</td>
<td>0.007 ± 0.001**</td>
</tr>
<tr>
<td>Normal (n = 12)</td>
<td>21.62 ± 0.84</td>
<td>0.012 ± 0.001</td>
</tr>
</tbody>
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*p<0.01, as compared to animals receiving water ad libitum.

**p<0.05, as compared to animals receiving water ad libitum.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>24-h food intake (g/g body mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>Water-restricted</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Aspermic</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Normal</td>
<td>0.18 ± 0.01</td>
</tr>
</tbody>
</table>
TABLE 3. Daily average water intake before and after water restriction.

<table>
<thead>
<tr>
<th>Daily average water intake (cc/g body mass)</th>
<th>Ad libitum</th>
<th>25% of Ad libitum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>0.33 ± 0.02</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>Water-restricted</td>
<td>0.34 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Aspermic</td>
<td>0.30 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.33 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Normal</td>
<td>0.39 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
</tbody>
</table>

sometimes associated with limited water availability (MacMillen, 1963; 1983; King, 1968). The present array of reproductive responses induced by water restriction are probably associated with a number of functional changes in one or more organ systems that maintain water balance during periods of reduced water intake. While the full set of functional adjustments remains to be identified, it is possible that differential hydrolysis of body fat reserves (Guyton, 1986) could account for the different spermatogenic responses to water restriction. The adaptive value of these adjustments is to allow the animals to survive during poor environmental conditions.

The ecological implications of our findings are intriguing, since only a fraction of the animals respond reproductively to reduced water intake. Perhaps other populations of deer mice from more arid or xeric habitats might exhibit greater gonadal responsiveness to water restriction than mice indigenous to the mesic regions of South Dakota. Previous work on species from xeric habitats suggests that voles, paradoxically, display reduced responsiveness to decreased water intake (i.e., maintenance of gametogenesis; e.g., Lidicker, 1976; Nelson et al., 1983). The important ecological aspect of our results is that testicular regression induced by limited water availability is independent of other cues known to control reproductive performance, namely, photoperiod, temperature, and food. Despite variation in the responsiveness to water, the present work suggests that restricted water consumption may act to arrest reproduction during the peak of the annual breeding season in some individuals of this deer mouse subspecies. Limited food availability during these times may be secondary to restricted water intake to stop breeding in the middle of the season.

In conclusion, our data add another level of control in the environmental contingencies that influence reproductive success. Photoperiodic cues allow animals to forecast a generally optimal reproductive season. However, animals attuned to food or water availability can override favorable day length or temperature information and more closely monitor local conditions and precisely time breeding (cf. Negus and Berger, 1972; Zucker et al., 1980). We propose that arrested reproductive competence due to limited water availability is part of a physiological adaptation that allows animals to survive a summer drought.

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