Cytochrome Oxidase Activity in Brown Fat Varies With Reproductive Response and Use of Torpor in Deer Mice

JAMES L. BLANK,* RANDY J. NELSON† AND ASTRID BUCHBERGER‡§

*Department of Biological Sciences, Kent State University, Kent, OH 44242
†Department of Psychology and Population Dynamics, Johns Hopkins University, Baltimore, MD 21218
‡Fachbereich Zoologie, Philipps Universitädt, D-3550 Marburg, Federal Republic of Germany
§Institut für Humangenetic, fuer Universitäts-Klinik Eppendorf, Bufeufeld 32, 2000 Hamburg 54
Federal Republic of Germany

Received 28 September 1987

BLANK, J. L., R. J. NELSON AND A. BUCHBERGER. Cytochrome oxidase activity in brown fat varies with reproductive response and use of torpor in deer mice. PHYSIOL BEHAV 43(3) 301-306, 1988.—Reproductive responses and thermogenic properties of brown adipose tissue (BAT) were evaluated in individuals from an outbred population of deer mice (Peromyscus maniculatus nebrascensis) after 10 weeks exposure to short photoperiod (8:16 light:dark) and cold ambient temperature (2°C). Deer mice populations are composed of phenotypes that differ in their reproductive response to environmental cues. These phenotypes also differ in body temperature regulation as indicated by their use of daily torpor. By comparing BAT responses among individuals of different phenotypes, we were able to assess the association between environmentally induced changes in reproduction and metabolism. Short/cold days caused increased proliferation of BAT and higher cytochrome oxidase activity. However, the magnitude of these changes varied with reproductive phenotype and use of daily torpor. BAT weight in short/cold day exposed males with normal sized testes more than doubled while total cytochrome oxidase activity increased by 30% as compared to controls. In contrast, short/cold day exposed deer mice with atrophic testes that employed daily torpor exhibited a 64% increase in BAT weight and 100% increase in total cytochrome oxidase activity, compared to control mice. Cytochrome oxidase activity in nontorpid deer mice with atrophic testes was intermediate to these two groups. Our results demonstrate a response of BAT to short/cold days that varies with individual reproductive response. This finding suggests that there exists a common integrative mechanism for temperature and photoperiod to regulate both seasonal reproductive and metabolic adjustments.

Brown adipose tissue Male reproduction Photoperiod Seasonal breeding Torpor Deer mice

Day length

AMONG the adaptations mammals make in response to the winter environment is the neurally directed involution of the reproductive system. This process is initiated by specific proximate signals that are predictive of changes in an animal’s environment. Stimuli such as photoperiod [9], ambient temperature [5], food [1,7] and water [20,21] are perceived by the individual via numerous sensory inputs and ultimately integrated by neurosecretory centers in the anterior hypothalamus [4,10]. Photoperiod-induced changes in the secretion of hypothalamic releasing factors by these neurosecretory centers in turn modify production and secretion of pituitary gonadotrophins [2, 4, 25, 26]. Alterations in the pattern of hormonal release from the pituitary are responsible for subsequent cessation of gonadal function and elimination of all sex behavior.

The selective benefit to the individual in turning off the reproductive system during the winter has been proposed to lie in the elimination of offspring production during a time of the year unfavorable for their survival [26]. Conceptually, allocation of energy to reproduction would be wasted when the probability of offspring survival is low. Another benefit from the elimination of breeding would be the ability to preferentially allocate energy to other physiological and behavioral activities that increase parental survivorship.

In the context of survival value the cessation of reproduction is by no means the only or even the most obvious physiological adjustment animals make on a seasonal basis. Many winter environments subject small mammals to ambient temperatures far below thermoneutrality, a condition that significantly increases loss of body heat to the environment. This energy loss is especially acute in small rodents that have a large surface to volume ratio and must be balanced by equivalent energy gains if the animal is to survive. Since winter environments are typically characterized by re-
duced food availability, animals must increase metabolic heat production during the period of lowest fuel availability. Consequently, animals must make seasonal metabolic adjustments that extend from the cellular to the whole organism level of biological organization to cope with this metabolic challenge [13,27].

A temporal association of seasonal metabolic and reproductive responses within the individual is known to occur in several species [3,17]. Recent work with male deer mice (Peromyscus maniculatus) shows that the reproductive axes of individuals derived from the same natural breeding population respond differentially to inhibitory environment cues [2,5]. Pituitary-testicular function is arrested in about one-third of all males exposed to short photoperiods, but an equal number of males remain fertile. This phenotypic difference is now known to have a genetic basis [6]. When individuals of these two genotypic groups are further examined for their metabolic response to inhibitory environmental signals, they are found to exhibit individual differences in body temperature regulation. This association suggests that reproductive genotypes in this species possess different metabolic adaptations to the same environment [2].

In the present study we examined the response of a specific metabolic parameter also related to body temperature regulation, cytochrome oxidase activity in brown adipose tissue (BAT), in relation to gonadal response to photoperiod and ambient temperature. BAT affects body temperature through its production of heat, and serves to increase or maintain body temperature in cold environments. This function of brown fat cells, termed nonshivering thermogenesis (NST), is conferred via a special mitochondrial shunt that releases the energy from the breakdown of substrates as heat instead of fixing this energy into molecules of ATP [22]. Short photoperiod and cold ambient temperature may both affect the structure and function of BAT [13]. This suggests that in certain species the function of this metabolic tissue is regulated by neuroendocrine processes similar to those that regulate seasonal involution of the gonad.

Our results provide further evidence of a patterned association between seasonal metabolic and reproductive adjustments. These data demonstrate that BAT of deer mice is modified by photoperiod and/or ambient temperature. Furthermore, the type of modification that occurs differs among the reproductive phenotypes. Together, these data suggest the existence of a common integrative neuroendocrine center for reproductive and metabolic responses to photoperiod and ambient temperature.

**METHOD**

**Animals**

Deer mice (Peromyscus maniculatus nebrascensis) were selected from the F2 offspring of an outbred F1 breeding stock. The parental generation of the F1 was captured in the vicinity of Wind Cave National Park, Hot Springs, SD (43° 30’N Lat; 103° 34’W Long). The breeding methods employed for the F1 produced an outbred experimental stock with an average genetic relatedness of 0.125 [2].

Male offspring were weaned at 21 days of age and housed singly in polypropylene cages and provided with food (Formulab, Purina, St. Louis, MO) and water ad lib. All males selected for the study were reproductively mature at 60 days of age as assessed by external measurement of the testis (length × width). Males with testis size of 54 mm² or larger successfully impregnate females and were judged reproductively competent for this study [5].

**Experimental Conditions**

Following continuous exposure from birth to long day length (16 L:8 D) and warm ambient temperature (23°C), hereafter long/warm days, males were transferred to short photoperiod (8 L:16 D) and cold ambient temperature (2°C), hereafter short/cold days, for 10 consecutive weeks. A group of males maintained on long/warm days served as controls (n=10). At the end of the experimental period, all animals were weighed and sacrificed. Paired testes were removed and weighed.

For comparison of BAT characteristics, deer mice were divided into three groups on the basis of two characteristics. First, males were grouped on the basis of external measurement of testis size; these groups were, (1) normal males whose testis size after 10 weeks exposure to short/cold days was equal to or greater than long/warm day exposed controls and (2) atrophic males whose testes were equal to or less than the minimum testis size of reproductively incompetent males, as published elsewhere [5]. Following this categorization, we further characterized these deer mice for their use of daily torpor. This behavior is characterized by a daily lowering of body temperature to levels below 20°C. Such deep torpor is readily determined by visual examination and rectal measurement of body temperature [11]. We determined the presence or absence of daily torpor of individual deer mice during the 10th week of exposure to inhibitory environmental conditions by these two methods. Previous work had shown that only 25% of deer mice with atrophic testes exhibit daily torpor [3]. The same observation was made in this experiment.

Thus, BAT response to short/cold days was compared in three groups of deer mice: (Group 1) males with normal testis size that did not display daily torpor (n=9), (Group 2) males with atrophic testes that did not display daily torpor (n=11), and (Group 3) males with atrophic testes that displayed daily torpor (n=12).

**Brown Adipose Tissue**

Response of BAT to short/cold days was assessed in the interscapular brown fat pad. This pad was removed from animals immediately after they were sacrificed, and trimmed of connective tissue, white adipose tissue, and skeletal muscle. Each fat pad was then weighed and immediately snap-frozen on dry ice.

Cytochrome oxidase activity of BAT was measured as an index of mitochondrial capacity. Activity was determined polarographically in tissue homogenates at 25°C using a Clark electrode (Bachofer, Rentlinger, Federal Republic of Germany). Tissue preparations were diluted in 0.5% Lubrol WX (Sigma, St. Louis, MO). Further details are described elsewhere [22]. The activity of cytochrome oxidase is given in Units (U) where 1 U equals 1 μmol O₂ consumed per min at 25°C.

**Statistical Analyses**

All values are expressed as mean ± 2 standard error for mice housed on each environmental treatment. Main treatment effects were evaluated with a one-way ANOVA and group differences by the Tukey range test.
TABLE 1
PAIRED TESTES WEIGHT AND BODY WEIGHT FOR DEER MICE EXPOSED TO EITHER LONG/WARM DAYS (16 L:8 D, 23°C) OR SHORT/COLD DAYS (8 L:16 D, 2°C) FOR 10 WEEKS

<table>
<thead>
<tr>
<th></th>
<th>16 L:8 D</th>
<th>Large Gonads</th>
<th>8 L:16 D</th>
<th>Small Gonads</th>
<th>Small Gonads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23°C</td>
<td>No Torpor</td>
<td>2°C</td>
<td>No Torpor</td>
<td>Torpor</td>
</tr>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=9)</td>
<td>(n=11)</td>
<td>(n=12)</td>
<td></td>
</tr>
<tr>
<td>Paired Testes</td>
<td>320.67</td>
<td>328.07</td>
<td>73.25</td>
<td>73.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(9.6)</td>
<td>(7.6)</td>
<td>(2.2)</td>
<td>(4.4)</td>
<td></td>
</tr>
<tr>
<td>Body Weight</td>
<td>25.62</td>
<td>26.47</td>
<td>23.95</td>
<td>21.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.80)</td>
<td>(0.82)</td>
<td>(0.80)</td>
<td>(0.46)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are mean ± 2 S.E.M. Statistical comparisons are provided in text.

RESULTS

Reproductive Responses

Deer mice responded to short/cold days with a characteristic array of reproductive responses. Testicular mass differed significantly among those mice chosen for comparison of BAT characteristics (Table 1). The presence or absence of torpor among mice with atrophied testes was not associated with any difference in gonad mass (Table 1).

Body weight differed among the 3 subsets of short/cold day exposed deer mice (Table 1); no differences were observed among these same mice prior to treatment (Table 1). Among short/cold day mice with atrophied testes, body weight differed according to the use of torpor. Deer mice which displayed torpor exhibited a mean body weight significantly less than that of all other treatment groups (p < 0.05). Mean body weights of short/cold day deer mice with atrophied testes that did not exhibit torpor and short/cold day males with normal sized testes did not differ significantly from one another (p > 0.05) or from controls (p > 0.05).

Brown Adipose Tissue Responses

Short photoperiod and cold ambient temperatures had a significant effect on both BAT weight and cytochrome oxidase activity. Interscapular BAT weight increased significantly in all 3 groups of deer mice (Fig. 1). However, the magnitude of response differed significantly among testicular and torpor subsets (Fig. 1). Mice with normal testes exhibited the greatest increase in absolute BAT weight, about 2.5 times that of controls (p < 0.05). The small increase in BAT weight among mice with atrophied testes was also significant (p < 0.05) as compared to control values; the presence or absence of torpor had no differential effect on BAT proliferation in these two groups.

When body weight differences are taken into account, the relative proliferation of BAT among all 3 groups differs from the above pattern (Fig. 2). Again, all subsets exhibited a significant increase in weight of the interscapular BAT (p < 0.05 in all cases). But deer mice with atrophied testes that employed torpor exhibited the greatest relative response and males with normal testes the least.

The response of BAT as measured by cytochrome oxidase activity did not parallel changes noted in absolute BAT weight (Figs. 3, 4). When cytochrome oxidase activity is expressed as U/g tissue (Fig. 3), only mice with atrophied testes exhibited a significantly greater activity compared to long/warm day exposed controls (p < 0.05). Cytochrome oxidase activity increased significantly in both groups of mice with atrophied testes (p < 0.05), regardless of their use of torpor. In contrast, cytochrome oxidase activity in deer mice with normal-sized testes was nearly identical to controls (p > 0.05).

Because each deer mice subset exhibited variability in the hypertrophy of interscapular BAT weight, there were significant differences between groups in total cytochrome oxidase activity (Fig. 4). In all 3 short/cold day exposed
FIG. 2. Interscapular BAT pad weight as a percent of body weight. Subsets and environmental conditions are as described in Fig. 1; bars are mean ± 2 S.E.M. Relative BAT pad weight increased significantly (p<0.05) in all mice exposed to short/cold days. Relative BAT pad weight in atrophic deer mice that also displayed torpor was greater than in other short/cold day subsets (p <0.05). Relative BAT pad weight in nontorpid males with large and small gonads did not differ significant from one another (p >0.05).

FIG. 3. Cytochrome oxidase activity in adipocytes of interscapular BAT, expressed as units per gram tissue. Subsets and environmental conditions are as described in Fig. 1; bars are mean ± 2 S.E.M. Individuals with small gonads following exposure to short/cold days exhibited a significant (p<0.05) weight-specific increase in activity; animals with large gonads did not differ significantly from long/warm day controls (p >0.05). The occurrence of torpor had no effect on cytochrome oxidase activity.

FIG. 4. Cytochrome oxidase activity in adipocytes of interscapular BAT, expressed as total units per pad. Subsets and environmental conditions are as described in Fig. 1; bars are mean ± 2 S.E.M. All short/cold day groups showed a significant (p<0.05) increase in total units of activity per BAT pad. Activity in nontorpid males with large and small gonads did not differ from one another (p >0.05), and exhibited a 30% and 40% increase in activity, respectively. Atrophic males that also used torpor had significantly (p<0.05) higher units of activity per pad than other short/cold day subsets.

DISCUSSION

Short photoperiods and cold ambient temperatures are two environmental cues that predict the onset of winter conditions at temperate latitudes. When deer mice derived from a naturally-selected population are exposed to these two factors, complete testicular regression occurs in about 30% of all animals [2]. An equal number of individuals remain reproductively competent and are capable of producing offspring. This general phenomenon of differential reproductive response to environmental cues occurs in other temperate-zone rodents [1, 15, 17, 20, 21]. Field studies also indicate that winter breeding is a frequent occurrence in a variety of species [16, 18, 19], demonstrating the presence in natural populations of individuals in which the reproductive system is not affected by inhibitory cues. Thus, variability in response to environmental factors typical among deer mice is probably widespread among rodent species. The present findings provide further evidence that male deer mice also exhibit individual variation in cytochrome oxidase activity of interscapular brown fat in response to the same environmental signals that affect the reproductive system. BAT responds to short/cold days in all individuals, but in varied patterns that also covary with the reproductive response and use of daily torpor.

There were also differential changes in BAT cytochrome oxidase activity. An increase in the activity of this enzyme
indicates an increase in mitochondrial protein and may indicate an increase in NST capacity [13]. Although NST was not assessed in these individuals, there was an increase in total cytochrome oxidase activity (U/pad) in all individuals regardless of reproductive response (Fig. 4). However, the magnitude of the increase differed significantly among reproductive and torpor phenotypes. Deer mice with atrophic testes that displayed torpor exhibited a 100% increase in total cytochrome oxidase activity (U/fat pad) compared to controls. In contrast, all other short/cold day mice with either atrophic testes or with normal testes showed an increase in total cytochrome oxidase activity of 40% and 30%, respectively.

The positive relationship between increased cytochrome oxidase activity and torpor has to our knowledge not been previously reported. It is not known whether this association also extends to the thermogenic ability of the brown fat. NST has been implicated as providing heat during arousal from hibernation in ground squirrels and marmots [13], although its significance in deer mice is unknown. Nevertheless, greater weight loss and higher cytochrome oxidase activity in nonbreeding deer mice using torpor suggests further phenotypic variation in seasonal metabolic adaptations.

The causes of elevated cytochrome oxidase activity also differed among the reproductive phenotypes. Males with normal testes possessed higher cytochrome oxidase activity because of an increase in BAT pad weight; cytochrome oxidase activity as a percent of body weight was unchanged (Fig. 3). In contrast, atrophic males possessed a greater proportional increase in cytochrome oxidase activity as a percent of body weight both because of a proliferation of the total pad (Fig. 1) and an increase in cytochrome oxidase activity per g BAT (Fig. 3). These data do not address the differential ability for NST in the phenotypes of deer mice. While a close correlation exists between NST and cytochrome oxidase in warm-adapted Djungarian hamsters, no such correlation exists in cold acclimated hamsters maintained on long day lengths [12]. The presence or absence of a correlation in short/cold day exposed deer mice is not known. Thus, we can make no conclusions about differences in the contribution of the interscapular fat pad to NST among the reproductive phenotypes.

Changes in gonadal function are only one of many adaptive physiological responses that take place on a seasonal basis. Regression of the gonads [24,26], use of daily torpor [3,13], body mass fluctuation [24], modification in structure and function of BAT [8, 13, 14, 23], tissue changes that affect heat flux of the body [13], and numerous behavioral responses integrated with biochemical changes [24] are among the constituent physiological responses. The data presented here and our interpretations emphasize two issues central to understanding the function and regulation of these physiological adaptations in winter environments.

The first issue rests on the question of adaptation. How do groups of physiological responses increase an individual's ability to adapt to its environment? Natural breeding populations of deer mice consist of phenotypes of individuals that possess sets of reproductive and metabolic traits. The structural and functional characteristics of these traits are not fully described within a single animal or within phenotypes. Presumably, these sets confer different advantages and disadvantages on the individuals that possess them in the context of differential survival and reproduction. The answer to this question of adaptation thus rests with a thorough description of how these phenotypes are able to adapt physiologically to the winter environment, and how the sets of physiological adjustments provide the means of adaptation.

The second issue relates to the question of the physiological regulation of these responses. Decreasing photoperiod and cold ambient temperature serve as two primary predictors of environmental change. These environmental cues act upon the central nervous system to trigger a set of neuroendocrine responses that cause both short- and long-term gonadal regression and metabolic acclimation. Hence, there is common cueing of both seasonal reproductive and metabolic changes by the same environmental stimuli. To what extent this commonality reflects an overlapping integrative center in the brain is unknown. The neural paths which detect and respond to light and elicit adjustments in gonadotrophic hormone secretion and gonadal function converge in the anterior hypothalamus. Likewise, many of the homeostatic aspects of thermoregulation including BAT characteristics and torpor are known to involve central and peripheral nervous paths, with the hypothalamus again serving to integrate multiple inputs into a coordinated metabolic response.

Thus, our results support the hypothesis that the neuroendocrine pathways that govern short/cold day induced changes in reproduction and metabolism converge at the level of the hypothalamus. It is not known whether the commonality in regulation occurs only at the neural level and involves the same neural substrates. However, the interactive role of the pineal and hypothalamus in mediating the photoperiodic and temperature effects on reproduction and metabolism suggests that the acclimation observed in each system is coordinated centrally. Another line of evidence for this hypothesis comes from previous studies which show that adjustments in BAT and appearance of daily torpor only occur in certain phenotypes of deer mice [3]. While this coincidence does not speak to the question of cause and effect, it suggests that the engagement of adjustments in one system is dependent upon engagement of adjustments in the other.

ACKNOWLEDGEMENTS

We thank Lester McClanahan and Richard Klukas of the U.S. Department of Interior, Wind Cave National Park, Hot Springs, SD, for their assistance in collecting parental stocks of deer mice. We also thank Sigrid St6hr for technical assistance and artwork. This research was supported by National Research Service Awards HD-06431 (J.L.B.) and HD-06587 (R.J.N.), and by an Alexander von Humboldt-Stiftung Research Fellowship (J.L.B.).

REFERENCES


