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Role of Melatonin in Mediating Seasonal Energetic and Immunologic Adaptations

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ABSTRACT: Winter is energetically demanding and stressful; thermoregulatory demands increase when food availability usually decreases. Physiological and behavioral adaptations, including termination of breeding, have evolved among nontropical animals to cope with the energy shortages during winter. Presumably, selection for the mechanisms that permit physiological and behavioral anticipation of seasonal ambient changes have led to current seasonal breeding patterns for many populations. In addition to the well-studied seasonal cycles of mating and birth, there are also significant seasonal cycles of illness and death among field populations of mammals and birds. Energetically challenging winter conditions can directly induce death via hypothermia, starvation, or shock; surviving these demanding conditions likely puts individuals under great physiological stress. The stress of coping with energetically demanding conditions may increase adrenocortical steroid levels that could indirectly cause illness and death by compromising immune function. Individuals would enjoy a survival advantage if seasonally recurring stressors could be anticipated and countered by bolstering immune function. The primary environmental cue that permits physiological anticipation of season is daily photoperiod, a cue that is mediated by melatonin. However, other environmental factors may interact with photoperiod to affect immune function and disease processes. Immune function is compromised during the winter in field studies of birds and mammals. However, laboratory studies of seasonal changes in mammalian immunity consistently report that immune function is enhanced in short day lengths. To resolve this apparent discrepancy, we hypothesize that winter stressors present in field studies counteract short-day enhancement of immune function. Prolonged melatonin treatment mimics short days, and also enhances rodent immune function. Reproductive responsiveness to melatonin appears to affect immune function. In sum, melatonin may be part of an integrative system to coordinate reproductive, immunologic, and other physiological processes to cope successfully with energetic stressors during winter. © 1997 Elsevier Science Inc.

KEY WORDS: Melatonin, Seasonality, Thermoregulation, Energetics, Metabolism, Photoperiod.

INTRODUCTION

Melatonin is an indole-amine hormone that is found throughout the animal kingdom [42,97,101]. Although several poorly supported claims about the physiological effects of melatonin have gained prominence recently, there are many well-documented physiological and behavioral effects mediated by melatonin. The best-known and probably best-studied biological function of melatonin is to provide annual day length information. Melatonin, encoding day length (photoperiod) information, appears to be the primary hormone orchestrating the seasonal changes in reproductive function observed among many mammals living in mid to high latitude habitats [6,40]. Melatonin is often considered primarily as a reproductive hormone [6,97]; it regulates the onset of puberty, as well as the seasonal pattern of reproductive function and quiescence. Melatonin also affects a wide range of seemingly unrelated physiological, morphological, and behavioral processes. In this review, we emphasize that melatonin is a hormone that integrates seasonal adaptations [62]. Many seasonal adaptations, including reproductive inhibition, increased thermoregulatory capacities, and enhanced immune function, have evolved to help animals cope with the annual changes in environmental energy demands. For example, melatonin appears to affect body mass regulation, gut efficiency, metabolic rate, pelage development, and noshivering thermogenesis (NST) [48,49,62,100,101]. In some species (e.g., *Dicristonyx groendlandicus*), melatonin regulates the winter development of special claws on the forelegs that aid in digging through the snow [41]. Melatonin also affects several energy-saving behaviors including nest-building, torpor, and food intake [6,61,93]. The inhibitory effects of melatonin on reproductive function also represent an energy savings adaptation—animals have been selected to forego breeding when reproductive success is unlikely. Presumably, reproductive regression reflects the energetic incompatibility of breeding (e.g., mating, lactating, resource defense) and thermoregulatory activities during winter. However, the existence of winter breeding in virtually every population studied indicates that this energetic incompatibility can be resolved.

Photoperiodic information is used to initiate or terminate specific seasonal adaptations, including reproduction, to maintain a positive energy balance (reviewed in [5,49,59,101]). The annual cycle of changing photoperiod is a very precise temporal cue for determining the time of year. Ambient photoperiodic information is transduced by the pineal gland into a melatonin signal; peak melatonin concentrations occur during the dark and basal levels occur during the light portion of the day. The secretory pattern of melatonin allows individuals to ascertain the time of year and thus anticipate predictable seasonal environmental changes (reviewed in [5,97]).

Although maintenance of a positive energy balance is critical for survival and reproductive success (reviewed in [22,82]), other

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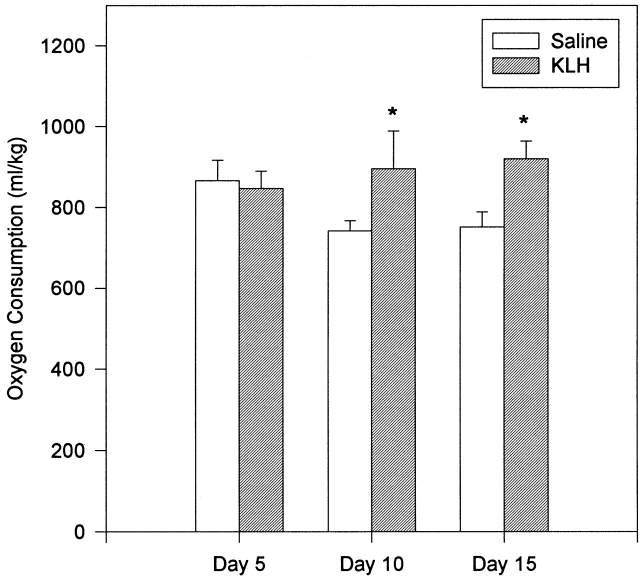

FIG. 1. Mean [±standard error of the mean (SEM)] oxygen consumption 5, 10, and 15 days after male house were injected SC with 150 μg of KLH suspended in 0. 1 cc sterile saline. Significant differences ($p < 0.05$) are indicated by the asterisk.

threats to survival must also be met for individuals to increase their fitness [84]. They must avoid predators and potentially dangerous interactions with conspecific competitors, as well as avoid succumbing to disease. Melatonin is critical in mediating the seasonal pelage color changes that are necessary for cryptic behaviors required by successful individuals of both prey and predator species [35,41]. Melatonin also mediates the seasonal decrease of steroidal hormones; low blood androgen concentrations reduce agonistic behaviors [81]. For example, the frequency of communal huddling, an energy-saving behavior, is increased among several rodent species during the winter (e.g., [62,73]), these rodents are highly aggressive and territorial during the spring and summer when sex steroid levels are high [82].

Immunological resistance also requires energy. In fact, the cascade of cellular events during the acute phase immune response and inflammation, and the elevation of body temperature in response to cytokine activation, presumably requires substantial energy, although precise quantification is lacking ([50,70], but see below). Cytokine activation elevates body temperature and the energy requirements of inflammation and acute phase immune responses may increase metabolic rates >10% per degree of body temperature elevation (reviewed in [69]). In a preliminary study of the energetic costs of mounting an immune response [33], house mice (*Mus musculus*) were injected with a specific antigen, Keyhole Limpet Hemocyanin (KLH). This substance induces an antibody response without inducing fever or making the treated animal sick [31]. Both oxygen consumption (ml/kg) and metabolic heat production (kcal/kg) increased in KLH-injected animals (Fig. 1). Thus, a general energy deficit can increase the risk of infection and death because insufficient energy reserves may be available to sustain immunity.

Stress compromises immune function (see [1,36,87] for reviews). Prolonged or severe food shortages may evoke secretion of glucocorticoid hormones [79]; glucocorticosteroids actively compromise aspects of immune function [53,70,78].

SEASONAL STRESSORS AND THE EFFECTS OF GLUCOCORTICOIDS ON IMMUNE FUNCTION

Many interactions between glucocorticoids and immune cell function have been reported in relation to environmental stress (reviewed in [78]). However, the mechanisms underlying seasonal changes in stress hormones and immune function have not been elucidated. Adrenocortical hormones, especially glucocorticoids, suppress immune function in both humans and nonhuman animals [1,7,11,27,45]. Glucocorticoids are released in response to stressful stimuli, and can compromise cellular and humoral immune function [8,56]. Adrenalectomy enhances lymphatic organ masses and B-cell activities [98]. The precise mechanisms by which the immune system is affected by the hypothalamic–pituitary–adrenocortical axis (HPA) are unknown, but probably involve cytokine release rates from activated immunological cells [9,10]. Regardless of mechanism, substantial evidence links glucocorticoids with suppressed immune function.

Recently, a direct link between melatonin and glucocorticoid biology has been established. Generally, melatonin enhances immune function, whereas glucocorticosteroids compromise immune function [43,66–68]. Melatonin treatment, however, can ameliorate the immunocompromising effects of glucocorticosteroids [2,3,14,65]. Conversely, glucocorticosteriods can reduce the immunoenhancing properties of melatonin. For example, cortisol treatment of ducklings reduced the number of thymic melatonin receptors [89].

Previous studies have demonstrated that environmental stressors elevate blood glucocorticoid levels and that high glucocorticoid levels suppress immune function [1,7,9,11,27,45,51]. For example, low ambient temperatures are often perceived as stressful, and can potentially compromise immune function (e.g. [27,65,77]). Winter survival in small animals is hypothesized to require a positive balance between short-day enhanced immune status and glucocorticoid-induced immunosuppression [30]. This immunosuppression may be due to many factors, including overcrowding, increased competition for scarce resources, low temperatures, reduced food availability, increased predator pressure, or lack of shelter. Each of these potential stressors may cause high blood concentrations of glucocorticoids. Winter breeding with its concomitant elevation in sex steroid hormones may also cause immunocompromise (e.g., [58,108]). Presumably, winter breeding occurs when other environmental stressors such as temperature and food availability are not severe. The balance of enhanced immune function (i.e., to the point where autoimmune disease becomes a danger) against stress-enduced immunosuppression (i.e., to the point where opportunistic pathogens and parasites overwhelm the host) must be met for animals to survive and become reproductively successful. Thus, the mediation of reproductive function and immune function will likely be intertwined [9].

Recently, the interaction between photoperiod and temperature was examined on antibody levels and splenic mass in male deer mice [30]. Animals were maintained in LD 16:8 or LD 8:16 photoperiods and either in 20° or 8°C temperatures. Serum immunoglobulin G (IgG) levels were elevated in short-day mice maintained at normal room temperature compared to long-day animals. Long-day deer mice kept at 8°C temperatures had reduced IgG levels; mice exposed to short days and low temperatures had IgG levels comparable to long-day mice maintained at 20°C. In other words, short days elevated IgG levels over long days. Low temperatures caused a significant reduction in IgG levels. The net effect of short-day enhancement and low temperature reduction of IgG levels is no appreciable difference from baseline (i.e., long-day mice kept at 20°C). This adaptive system may help animals

cope with seasonal stressors and ultimately increase reproductive fitness.

Many other conditions perceived as stressful, such as reduced food availability, low ambient temperatures, overcrowding, lack of shelter, or increased predator pressure, can recur seasonally leading to seasonal fluctuations in immune function among individuals, and seasonal changes in population-wide disease and death rates [58]. A dynamic relationship exists between longevity and reproductive fitness [107]; all other things being equal, longer lived individuals produce more offspring and are more fit than individuals that die early.

In addition to the well-established seasonal cycles of mating and birth, there are also seasonal cycles of illness and death among many populations of animals (e.g., [15,58,72,74]). Because many stressful environmental conditions are somewhat recurrent, we hypothesize that animals have evolved mechanisms to combat seasonal stress-induced reductions in immune function. From an evolutionary and ecological perspective, it is reasonable to expect that animals have evolved the ability to forecast recurrent conditions associated with immunosuppression and bolster immune function in advance of these challenging conditions to maximize survival.

Thus, individuals use photoperiodic information to bolster immune function in anticipation of challenging energetic conditions that may otherwise compromise immune function. Again, enhanced immune function is one component of a complex web of winter coping adaptations. Virtually all laboratory studies of photoperiodic effects on immune function have reported enhanced immune function in short day lengths (reviewed in [83,84]). Although many field studies support this hypothesis, with data suggesting enhanced immune function and decreased disease prevalence during the winter compared to the summer, a substantial number of studies have reported the opposite pattern of results [83,84]; i.e., immune function is coincident with the short days of winter. These conflicting results can be resolved by considering additional environmental factors, not usually manipulated in laboratory studies. For example, winter-associated stressors (e.g., restricted food and low ambient temperatures) appear to counteract short-day enhancement of immune function in the lab (reviewed in [30]). Thus, we predict enhanced immune function should be observed during mild winters, whereas compromised immune function should be expected during challenging winters. Long-term field studies are required to test this hypothesis. Although the effects of melatonin on immunity are well established (see [16] for recent reviews), an ecological context is needed to understand the effects of melatonin upon immune function, and to suggest why this phenomenon might be adaptive and functional, rather than merely a physiological oddity. Knowledge of the adaptive and functional significance of seasonal fluctuations in immune function may help to provide an improved understanding of the possibilities, as well as the constraints, of melatonin immunotherapy.

To examine the role of energetics in seasonal changes in immune function in deer mice, the chemical compound 2-deoxy-D-glucose (2-DG) was used to manipulate energy availability at the input end of the energetic equation (Demas, DeVries, and Nelson, unpublished data). 2-DG is a glucose analog that inhibits cellular utilization of glucose, thus inducing a state of glucoprivation [105,114]. 2-DG acts as a metabolic stressor, increasing serum corticosterone levels [64], and 2-DG glucoprivation induces an anestrous state in female Syrian hamsters (*Mesocricetus auratus*) [102] and torpor in female Siberian hamsters (*Phodopus sungorus*) [29]. 2-DG induced metabolic stress also affects immune function; 2-DG administration inhibits murine splenic T lymphocyte proliferation in a dose-dependent fashion in laboratory strains of rats (*Rattus norvegicus*) [64] and mice (*M. musculus*) [75].

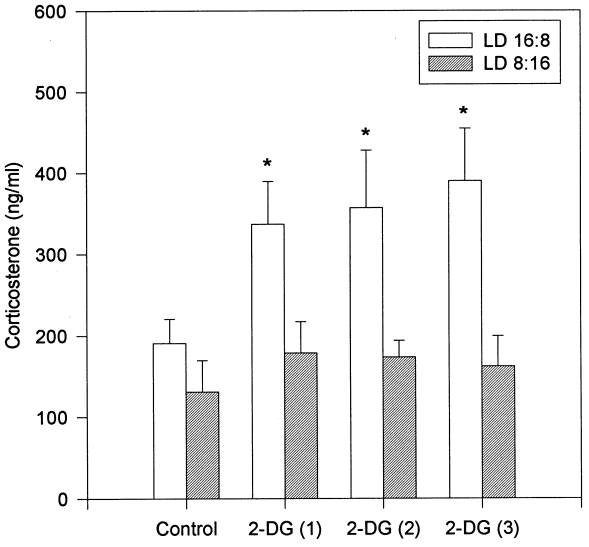

FIG. 2. Mean (±SEM) serum corticosterone (ng/ml) in deer mice housed in long (LD 16:8) or short (LD 8:16) days. Control mice in each photoperiod received daily IP injections of sterile 0. 9% saline across 3 consecutive days. Experimental mice received daily injections of 2-DG across 1, 2, or 3 consecutive days. Mice were lightly anesthetized with methoxyflurane vapors (Metofane, Pitman–Moore, Mudelein, IL), weighed, and blood samples obtained from the retro-orbital sinus. Handling time was kept constant and to a minimum; the time from initial removal from the cage to the end of bleeding was <3 min. Blood serum corticosterone levels were determined by radioimmunoassay using the ICN Biomedicals, Inc. ^{125}1 kit. This assay is highly specific, crossreacting at less than 0.3% with other steroid hormones. Intraassay variation was <4.5% Statistically significant differences between means are indicated by an asterisk.

In our preliminary study, short days buffered the animals against glucoprivation stress. Long-day mice injected with 2-DG had elevated corticosterone levels, as compared to long-day mice injected with saline (Fig. 2); corticosterone levels were not significantly elevated in short-day mice injected with 2-DG. 2-DG-treated long-day mice displayed reduced splenocyte proliferation to Con A as compared to control mice (Fig. 3). Splenocyte proliferation did not differ among short-day deer mice regardless of experimental treatment; short-day animals exhibited enhanced immune function; short-day mice treated with 2-DG displayed higher splenocyte proliferation than long-day mice treated with 2-DG (Fig. 3).

These data are also consistent with the hypothesis that short days buffer against metabolic stress. Reduced corticosterone levels in animals maintained on short days or treated with melatonin are likely due to improved metabolic function [101]. Accordingly, improved immune function in short days represents one component of numerous winter-coping adaptations that are mediated by melatonin.

WINTER BREEDING

Among long-day breeders such as rodents, so-called ''out-of-season'' breeding occurs in virtually every population examined, suggesting that the energetic bottleneck during winter can be resolved for small animals [52,54,80]. A substantial proportion of individuals not responsive to photoperiod has been reported to exist within every population of photoperiodic animals studied in the lab (e.g., [28,34,37,46,47,55,58,62,103,106,116]). There is a genetic basis for this variation because nonresponsiveness to pho-

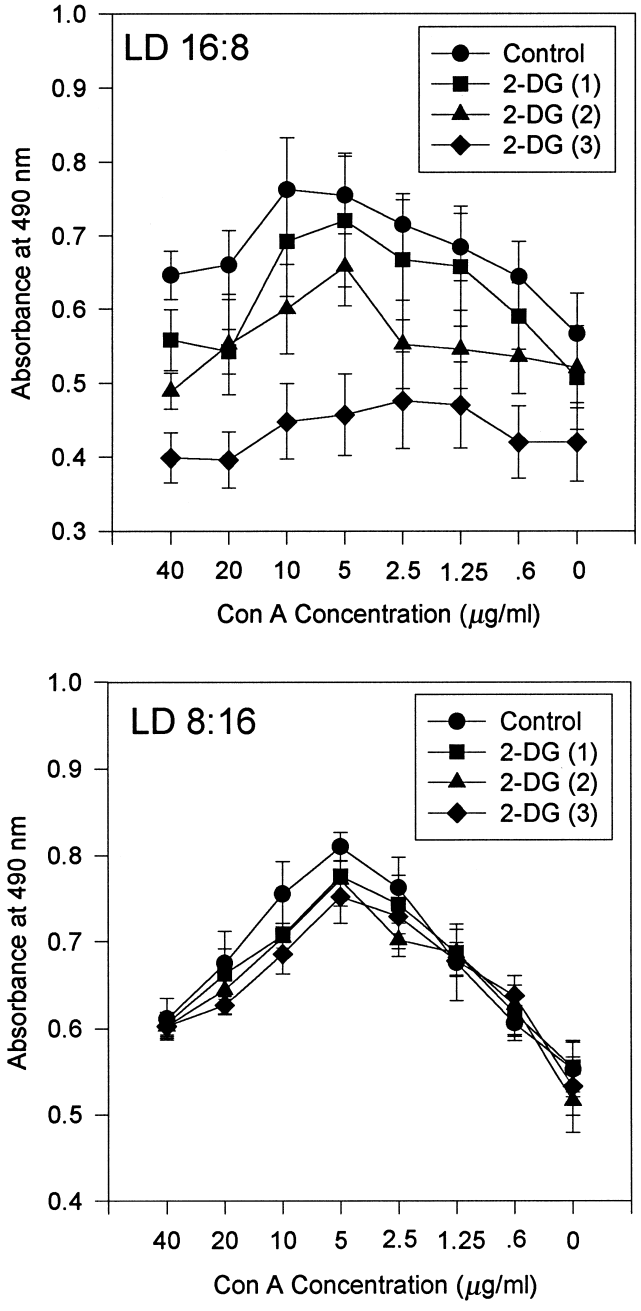

FIG. 3. Mean (±SEM) splenocyte proliferation to different concentrations of Con A (represented as absorbance units) of deer mice housed in (a) long (LD 16:8) or (b) short (LD 8:16) days. Control mice in each photoperiod received daily IP injections of sterile 0. 9% saline across 3 consecutive days. Experimental mice received daily injections of 2-DG across 2 or 3 consecutive days. Spleens were removed under aseptic conditions and suspended in culture media (RPMI-1640/HEPES). Proliferation was determined using a colormetric assay based on the tetrazolium salt 3-(4,5-demethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium. Higher absorbance values (nm) are indicative of increased splenocyte proliferation in response to mitogenic stimulation.

toperiod is a trait that can be selected (e.g., [47,54,63]). Despite the growing recognition that variation in responsiveness to photoperiod exists, there is currently no explanation for this variation on a physiological level. Nonresponsive morphs do not vary in their pineal melatonin content, melatonin secretion patterns, brain melatonin receptor numbers, or melatonin receptor binding affinities [12,24,47,113]. Exogenous melatonin treatment can induce reproductive regression in some nonresponsive individuals, but is ineffective for other species (see [4] for review). Nonresponsive phenotypes of Djungarian hamsters have asynchronous circadian rhythms [93,94], but individuals of other nonresponsive species display no obvious changes in circadian organization (e.g., [24,47]). Many tropical species do not display reproductive responsiveness to short photoperiod. For example, tropical cane mice (*Zygodontomys brevicauda*) do not inhibit reproductive function in response to any number of extrinsic manipulations or even in response to pharmacological treatment with melatonin [47]. Reproductive responsiveness to short days varies within populations from 0% (tropical species) to nearly 100% (boreal species) [28,61].

The circumstances that shift the cost-benefit ratio to favor winter breeding among individual small mammals remain unidentified. The existence of winter breeding is evidence that these circumstances exist. Animals that manage to breed successfully should increase reproductive fitness over that of nonbreeding conspecifics. However, this hypothetical increase in reproductive fitness will only accrue if the costs of winter-born offspring do not compromise survival [107]. Because not all animals breed during the winter, it is reasonable to surmise that there must be significant costs associated with winter breeding. Otherwise, the genes allowing winter breeding would spread and dominate in the population [44]. Some of these costs may reflect the survival costs associated with compromised thermogenic ability when breeding [109], whereas other costs may reflect energy demands that may not be met if energy resources are scarce.

The cost of reproduction for female mammals is high. For example, energy consumption often doubles during pregnancy and triples or quadruples during lactation [20,22]. The energetic savings for females to stop breeding when conditions are energetically challenging seem apparent. Females breeding out of season often perish [38]. However, the costs and benefits of seasonal reproductive quiescence to individual males are not obvious. Because males have evolved to exploit females' relatively high investment in offspring (e.g., [99]) they ought to maintain reproductive function throughout the year to take advantage of any mating opportunities.

To determine the costs and benefits of winter breeding, one study examined the extent to which male prairie voles (*Microtus ochrogaster*) and deer mice (*P. maniculatus*) that maintained ''summer-like'' reproductive systems when housed in winter-simulated day lengths also maintained other winter-coping adaptations. Circadian locomotor activity patterns, basal metabolic rate, capacity for NST, nest building, body mass, and daily food consumption were compared among short-day regressed males, short-day nonregressed males, and long-day males. Short-day deer mice that did not inhibit reproductive function resembled long-day males and differed from short-day conspecifics that inhibited reproductive function in body mass and nest-building behaviors [76]. Regardless of their reproductive response to photoperiod, short-day prairie voles reduced their daily food intake and wheel-running activity, but not body mass, compared to long-day voles [76]. These results suggest that winter breeding has energetic costs, most likely resulting from maintaining a ''summer-like'' body mass relative to that of reproductively regressed animals. These costs may be ameliorated to some extent by the reduction in locomotor activities and nest-building behaviors that seem tightly linked to photoperiod. In other studies, short-day voles that maintained reproductive function, retained photoperiodic responsiveness in other parameters; plasma prolactin levels and pelage de-

velopment were indistinguishable from short-day animals with regressed reproductive function [85,104].

PHOTOPERIODIC CHANGES IN IMMUNE FUNCTION

Laboratory strains of rats (*Rattus norvegicus*) are usually not considered reproductively responsive to photoperiod [86]. Nevertheless, adult Wistar male rats housed in constant dark (DD) for 4 weeks increased thymic mass by 315% over rats maintained in an LD 12:12 photoperiod; most of the increase was observed in the lymphatic tissue within the thymic medulla [69]. The number of thymocytes also increased in DD animals. Rats maintained for 4 weeks in constant bright light (LL) decreased thymic mass to 53% of values of LD 12:12 rats; the reduction in total volume represented mainly reductions in the thymic cortex [69]. Because photoperiod does not affect steroid hormones in male rats [86], these data strongly suggest that melatonin acts directly upon immune function [65]. Previous studies on rats have indicated slight photoperiod-induced changes in splenic weight [115]. Laboratory strains of house mice (*Mus musculus*) also display seasonal rhythms of immune function despite insignificant reproductive response to photoperiod [19,96].

Short days are effective in mediating immune function in individuals with robust reproductive responses to photoperiod [18]. For instance, splenic weights of deer mice (*Peromyscus maniculatus*) [112], and Syrian hamsters (*Mesocricetus auratus*) [17] were reduced in short days. Splenic masses, total splenic lymphocyte numbers, and macrophage counts were significantly higher in hamsters exposed to short days, compared to animals exposed to long photoperiods [17,18]. Photoperiodic influences on lymphocyte number and total white blood cell count have been reported for deer mice [13]; short day (LD 8:16) mice possessed more white blood cells than animals maintained in long day lengths (LD 16:8). More recently, short-day deer mice displayed faster healing rates, higher splenic T-lymphocyte proliferation, and higher specific antibody production than long-day mice [30,31,84].

EFFECTS OF MELATONIN ON IMMUNE FUNCTION

The pineal gland and its primary secretory pineal product, melatonin, affects lymphatic tissue size. For example, exposure of male and female hamsters to short days or daily afternoon melatonin injections increased splenic mass, which could be prevented in short-day hamsters by pinealectomy [110]. Importantly, melatonin mediates immune function [68]. In virtually all cases examined, melatonin enhanced humoral- and cell-mediated immunity [39,42,66]. Melatonin treatment of both normal and immunocompromised house mice elevated in vitro and in vivo antibody responses [25,26,64], restored impaired T-helper cell activity in immunocompromised mice [25], enhanced antigen presentation by splenic macrophages to T-cells [89], increased major histocompatibility (MHC) class II molecules, as well as IL-1 and tumor necrosis factor (TNF_{α}) production [89]. Pinealectomy reduced murine antibody-dependant cellular cytotoxicity (ADCC) [111].

As predicted [42,66] melatonin receptors have been isolated on circulating lymphocytes, thymocytes and bursa of Fabricius [3,57,59,60,71,87,88,92]. The melatonin receptors on lymphatic tissue appear similar in K_d values to melatonin receptors localized in rat and hamster brains, and also seem to be coupled to G-protein(s) [23]. Melatonin partially inhibited cyclic AMP production in human lymphocytes, but only at pharmacological doses [95].

In summary, melatonin appears to enhance immune function in most cases. In common with reproductive responses mediated by melatonin, there may be a temporal component to the biological

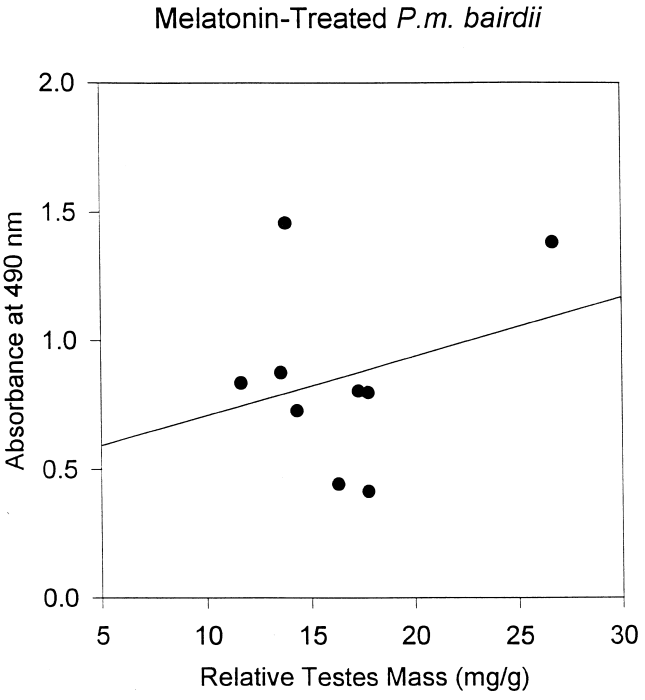

FIG. 4. Correlation of testes size and splenocyte proliferation to different concentration of Con A (represented as absorbance units) of deer mice implanted for 8 weeks with melatonin.

actions of this indole-amine. Most studies of melatonin effects on immune function have used animals that are not particularly responsive to this hormone (e.g., laboratory rodent strains) and may mask the immunoenhancing features of melatonin.

To what extent does immune function correlate with reproductive responsiveness to photoperiod? Among individual short-day deer mice that showed elevated splenic lymphocyte proliferation, there was no relationship between the degree of testicular regression and the amount of splenocyte proliferation (Fig. 4) (Demas and Nelson, unpublished data). These results suggest that photoperiodic responsiveness of immune function is not linked to reproductive responsiveness to day length. The effects of photoperiod and melatonin treatment on reproductive and immune function were assessed in two subspecies of *Peromyscus maniculatus* from different latitudes of origin [31]. Short-day *P.m. bairdii* (latitude = 42°51′ N) displayed reproductive regression and elevated splenocyte proliferation in response to the T-cell mitogen, Con A, as compared to long-day mice. In contrast, *P.m. luteus* (latitude = 30°37′ N) did not undergo reproductive regression in short-days; individuals of this subspecies also failed to exhibit any increase in lymphocyte proliferation to Con A in short days. Other individuals of both subspecies were implanted with empty capsules or capsules that contained melatonin. Individual *P.m. bairdii* implanted with melatonin underwent reproductive regression after 8 weeks of treatment. Individuals of this subspecies also displayed elevated lymphocyte proliferation to Con A compared to mice implanted with empty capsules. Conversely, *P.m. luteus* implanted with melatonin did not undergo reproductive regression and displayed no significant changes in lymphocyte proliferation. These data suggest that reproductive photoperiodic responsiveness, and more specifically, reproductive responsiveness to melatonin, mediates short-day enhancement of immune function in deer mice. These data also imply that melatonin may not possess universal immunoenhancing properties, and suggest that reproductive and immune responsiveness to day

length are linked in these species. The effectiveness of melatonin to influence immune responses may be constrained by reproductive responsiveness to this indole-amine.

Few studies have reported the effects of photoperiod on immune function of short-day breeders (e.g., sheep, red deer). Humans may retain minimal reproductive responsiveness to day length [21]. The extent to which humans retain immunologic responsiveness to day length or melatonin remains unspecified. Melatonin appears to be part of an integrated system involved in coordinating reproductive, immunologic, and thermoregulatory processes. Additional studies are required to understand the interactions among these physiological processes.

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