PLASMA ANDROGEN LEVELS AND BODY WEIGHTS FOR BREEDING AND NONBREEDING MALE AMERICAN KESTRELS[1]

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Abstract. Plasma androgen levels (1981) and body weights (1980 and 1981) were determined weekly between April and September for breeding and nonbreeding captive male American Kestrels (*Falco sparverius*) paired with females. Irrespective of breeding group, males exhibited comparable values for and weekly changes ($P < 0.01$) in both characteristics, although androgen levels appeared to decrease later and more abruptly in the spring for breeding males than for nonbreeding males. Mean ($\pm$ SE) androgen levels were maximal ($\geq 2.73 \pm 0.76$ ng/ml) in April and May, decreased to minimal values ($\leq 0.30 \pm 0.05$ ng/ml) by the second week of June, and remained relatively low through the end of September. Relative to the nesting stages, androgen levels for the breeding males peaked during both courtship stages and decreased ($P < 0.05$) to near minimum values the first week the second clutch was incubated. Androgen peaks were not different ($P > 0.10$) from each other. The difference ($P < 0.01$) between maximal (April, May, and September) and minimal (June, July, and August) body weights of males averaged 19%.

Key words: Androgen; plasma; body weight; breeding; nonbreeding; American Kestrel.

INTRODUCTION

For males of many avian species, testosterone induces aggressive, courtship, and sexual behavior, and plays important roles in spermatogenesis and the development of secondary sex characteristics (Wingfield and Farner 1980). Seasonal changes in blood testosterone levels in males of wild avian species have been reported for both free-living (Lisano and Kennamer 1977; Wingfield and Farner 1978a, 1978b, 1979; Berry et al. 1979; Burger and Millar 1980; Lincoln et al. 1980; Schwabl et al. 1980; Dittami 1981; Silverin and Wingfield 1982; Dawson 1983; Wingfield 1984; Hegner and Wingfield 1986) and captive (Kerlan and Jaffe 1974, Jallageas et al. 1978, Donham 1979) birds. The "Challenge Hypothesis" has recently been proposed (Wingfield et al. 1987) to explain these substantial seasonal changes since testosterone levels appear to be largely dependent upon the degree of male-male competition exhibited by the species (i.e., mate guarding and territoriality) and on the amount of parental care provided by the male.

Recently, we reported seasonal plasma hormone profiles for breeding and nonbreeding captive female American Kestrels (*Falco sparverius*) (Rehder et al. 1986). To our knowledge, this was the first plasma hormone data published for a raptor. The present study reports seasonal changes in plasma androgen level and body weight for breeding and nonbreeding captive male kestrels, including unreported data on those males paired with females in our earlier study. This provides the first information on blood androgen level for a wild altricial species breeding in captivity where life histories can be completed and blood-sampling regimes controlled.

MATERIALS AND METHODS

On 24 April 1980 and 8 April 1981, 10 American Kestrels of both sexes were randomly selected from the McGill University pedigreed colony (Bird 1982). Birds in 1981 were yearlings while those in 1980 varied from 2 to 6 years of age. Opposite sexes were randomly paired and placed in juxtapositional indoor polyethylene pens measuring 3.6 × 0.9 × 2.3 m and containing a nest box. Photoperiod and air temperature were maintained close to outdoor conditions (45°30′N, 73°26′W). Four days after a female had laid the last egg of her first clutch, the clutch was removed to induce the laying of a second clutch (Bird 1982). Fledglings from second clutches were left in their respective pens until the parents had completed

molting (Willoughby 1966), at which point the study was terminated.

Blood samples (1 ml) were obtained weekly (9 April to 30 September 1981) from each bird by brachial venipuncture as described by Rehder et al. (1982). To minimize possible diurnal fluctuations in plasma androgen level (Balthazart 1976) and body weight (Clark 1979), pairs were randomly sampled between 09:00 and 12:00 before feeding. Body weight was determined for each bird after it was bled. Finally, each pair was observed (10-min period between 11:00 and 13:00) through a one-way glass window on 5 days during the week. Based on behavioral observations, one of the following reproductive states was assigned to each pair: no interaction, first courtship (Willoughby and Cade 1964), laying of first clutch, second courtship, laying of second clutch, incubation, feeding of nestlings, and care of fledglings.

Androgen levels in male plasma samples (100 μl) were determined in duplicate following validation of a direct-plasma, double-antibody radioimmunoassay procedure (Schanbacher and D'Occhio 1982, Sanford 1985). Plasma obtained from male kestrels in the nonbreeding season was stripped of endogenous steroids with charcoal and added to reference tubes to standardize plasma concentration within all assay tubes. The intra- and interassay coefficients of variation for four duplicate androgen determinations on a kestrel plasma sample with a mean (±SE) level of 1.74 ± 0.07 ng/ml were 13.8 and 9.1%, respectively. Minimum (95% initial binding) and maximum (25% initial binding) detectable androgen levels were 0.17 and 9.00 ng/ml, respectively. Percentage recovery of unlabelled testosterone (1 ng/ml) added to the kestrel quality control plasma sample was 104 ± 4% ($n = 6$). Given that the primary antiserum cross reacts with dihydrotestosterone (35%) and that the plasma dihydrotestosterone level for avian species can be as much as 200% of the plasma testosterone level (Wingfield and Farner 1980), our hormone values are viewed as representing total androgen.

Analysis of variance with adjustments for unequal sample sizes and repetitive sampling of experimental units was used to determine the statistical significance of differences in androgen level or body weight (Snedecor and Cochran 1976). The Student-Newman-Keuls test was used to locate significant differences between mean androgen levels for 1981 males from the onset of the study to 2 weeks after the second clutches were laid. Logarithms of androgen and body weight data were employed in these analyses.

RESULTS

In both 1980 and 1981, male kestrels could generally be identified as breeders ($n = 5$) or nonbreeders ($n = 3$). Breeders and their mates exhibited all eight reproductive states (i.e., laid two clutches and successfully fledged the second). Nonbreeders and their mates did not exhibit courtship behavior and produced either infertile eggs (1980) or no eggs at all (1981). Data are not presented for the remaining two males from each year who demonstrated intermediate degrees of reproductive success.

Weekly mean androgen levels for the 1981 breeders and nonbreeders are presented in Figure 1B. Although group differences were not significant ($P > 0.10$) during a given week, there was a significant ($P < 0.01$) difference in androgen level between weeks. Androgen levels were maximal in April and May, decreased to minimal values by the second week of June, and remained relatively low through the end of September. The timing of the eight reproductive states between 1981 breeding males varied by only 1 to 3 days. Weekly androgen levels for these males, normalized to the times of egg laying of mates, are illustrated in Figure 1A. Androgen levels during the two egg-laying periods were plotted first; these points also represent the mean dates that clutches were being laid (May 10 and June 3). Values for samples obtained at weekly intervals around these two points were then averaged and plotted. A significant ($P < 0.01$) decrease in androgen level occurred between the week before the second clutch was laid and the first week the second clutch was incubated. However, mean values for breeding males were not different ($P > 0.10$) from those for nonbreeders during comparable weeks.

Although weekly mean body weights did not differ ($P > 0.10$) between breeding and nonbreeding males during either 1980 or 1981, the mean weight of both groups together did vary ($P < 0.01$) between weeks during both years (Fig. 2). Weights were generally highest in April, May, and September and lowest from June through August. Differences in body weight during comparable weeks of the 2 years were not significant ($P > 0.10$). The decrease in body weight from maximal values averaged 19% for breeding and nonbreeding males in both years.
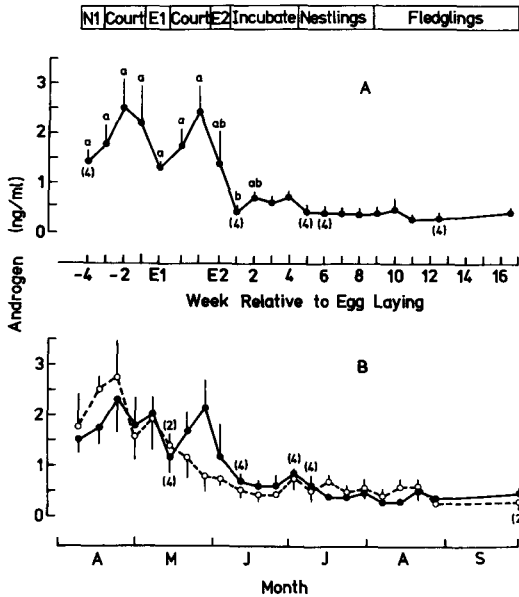
FIGURE 1. Mean (± SE) plasma androgen levels for 1981 breeding (closed circle) and nonbreeding (open circle) captive American Kestrel males for: (A) the weeks relative to egg laying and (B) the sampling weeks. Unless indicated otherwise in parentheses, each point represents a mean of five and three serially sampled breeding and nonbreeding males, respectively. Means with different letters are significantly (P < 0.05) different. N1 = no interaction; E1, E2 = females laying first and second clutches.

FIGURE 2. Weekly mean (±SE) body weights of 1980 and 1981 breeding (closed circle) (n = 5) and nonbreeding (open circle) (n = 3) captive American Kestrel males. N1 = no interaction; E1, E2 = females laying first and second clutches.

DISCUSSION

The direct-plasma assay validated for use in this study is a convenient and reliable method for measuring androgen levels in male birds when sample volume is not restrictive. Androgen levels for kestrels and assay reliability data obtained using this direct assay were comparable to those obtained for other breeding wild birds using more involved techniques (Kerlan and Jaffe 1974; Wingfield and Farner 1978a, 1978b, 1979; Burger and Millar 1980; Lincoln et al. 1980; Schwabl et al. 1980; Stokkan and Sharp 1980; Dittami 1981; Dawson 1983; Hegner and Wingfield 1986). In addition, given that the binding of labelled testosterone by the antiserum in our assay system was not affected by the volume of steroid-free plasma (50 vs. 100 µl per assay tube) and that lipid content in kestrel plasma does not appear to change with season (Henny and Meeker 1981), we believe that our results accurately reflect seasonal changes in plasma androgen level.
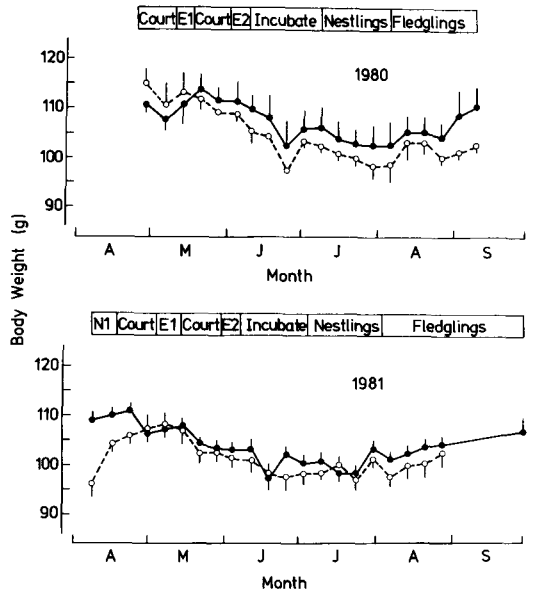
The observation that both nonbreeding and breeding male kestrels exhibited seasonal changes in plasma androgen level appears to be consistent with the Challenge Hypothesis (Wingfield et al. 1987). Androgen levels were maximal when males were involved in male-male competition for mates and territory, and minimal when males exhibited parental care. At the onset of this study, all males were photostimulated and provided with a female and nesting site in an attempt to induce breeding (Bird et al. 1980). Being a monogamous and territorial species, male kestrels encounter substantial competition among themselves in the spring. Since every male could hear and see silhouettes of the other pairs, all were presumably exposed to equal male-male competition. Not surprisingly, blood androgen levels for the breeding and nonbreeding males were comparable and maximal at the onset of the study while mates and territories were being established. After the first egg-laying period, androgen levels in both groups declined to similar values. It is believed that male-male competition was relatively low at this stage since females were no longer fertile (i.e., laying eggs) and territories had been established. Females regained their fertility, however, when first clutches were removed from the breeding pairs; this could have led to more male-male

competition for mates and explain the increase in androgen levels in the breeding males during their second breeding attempt. Since females paired with nonbreeding males were not fertile at this time, there was likely no resurgence in competition between these males. Consequently, an increase in blood androgen level in the nonbreeding males would not be expected. Androgen levels in the breeding males finally decreased to values seen for the nonbreeding males when the former began assisting with incubation.

Males of other monogamous territorial wild species which are normally single-brooded but renest (Wingfield and Farner 1979), have seasonal blood androgen profiles similar to those of the breeding kestrels in our study in that a pronounced androgen peak is associated with each clutch. Other avian species also exhibit the same relationship between reproductive status and androgen level as our kestrels. Mating and non-mating male captive Willow Ptarmigan, *Lagopus lagopus lagopus* (Stokkan and Sharp 1980), and free-living Canada Geese, *Branta canadensis* (Akesson and Raveling 1981), have similar maximal vernal blood androgen levels. In addition, prolongation of these seasonally high androgen levels in breeding vs. nonbreeding males has been demonstrated for Canada Geese (Akesson and Raveling 1981) and White-crowned Sparrows, *Zonotrichia leucophrys* (Moore 1983).

Decreases in blood androgen to minimal levels in both the nonbreeding and breeding male kestrels occurred at the time of estimated onset of photorefractoriness (Willoughby and Cade 1964). For most avian species (Feder et al. 1977; Paulke and Haase 1978; Wingfield and Farner 1978a, 1978b, 1979; Donham 1979; Burger and Millar 1980; Lincoln et al. 1980; Silverin and Wingfield 1982; Dawson 1983; Moore 1983; Rissmann and Wingfield 1984), blood androgen levels decrease to minimal or near minimal values at this time for reasons which remain speculative (Wingfield and Farner 1980).

Body weights for breeding and nonbreeding male kestrels changed throughout the breeding season. Similar seasonal weight profiles and percentage weight losses have been reported for males of wild breeding avian species (Wingfield and Farner 1978a, 1978b, 1979; Berry et al. 1979; Donham 1979; Lincoln et al. 1980; Dawson and Howe 1983; Wingfield 1984; Hegner and Wingfield 1986), including raptors (Hardy et al. 1981). Summer weight loss for the male kestrels did not appear to be related to the extra stress of breeding since males had similar weight profiles regardless of their breeding success. Others have also failed to establish a relationship between avian breeding success and body mass (Dittami 1981, Hegner and Wingfield 1987). The seasonal body weight profile for the male kestrel may reflect the negative correlation between air temperature and food consumption documented for this raptor (Craighead and Craighead 1956) and/or an adaptation to reduce the metabolic cost of flying during the nestling stage (Hegner and Wingfield 1987).

In conclusion, results of our study demonstrate that male kestrels exhibit seasonal changes in plasma androgen level and body weight regardless of their reproductive success. The duration of the springtime increase in androgen level is, however, influenced to some extent by their reproductive status.

ACKNOWLEDGMENTS

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