Relationships between Annual Cycles of Testosterone, Corticosterone, and Body Condition in Male Red-Spotted Garter Snakes, *Thamnophis sirtalis concinnus*

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ABSTRACT

Over a 2-yr period, we investigated the annual cycles of plasma testosterone and corticosterone and the relationships between these hormones and body condition in a wild population of male red-spotted garter snakes, *Thamnophis sirtalis concinnus*. In the 10 mo that were sampled, a peak in testosterone was observed in late summer during gametogenesis and declining through the spring breeding period. Corticosterone and testosterone cycles were positively correlated, in contrast to many vertebrates, suggesting the lack of a direct negative interaction between the two hormones. Body condition, defined as the residual of the regression of mass on snout-vent length, also cycled annually, with individuals being more robust during the summer than during the spring or fall. Individuals with a positive body condition had significantly lower plasma levels of corticosterone than did individuals with a negative body condition, supporting the energetic role of glucocorticoids. There was no relationship between body condition and testosterone. This study suggests that annual cycles of testosterone, corticosterone, and body condition can be associated with one another, and considering all three simultaneously is necessary to understand their control and function.

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Introduction

Understanding the seasonal cycles of steroid hormone secretion is a necessary prerequisite for conducting experimental manipulations to elucidate the behavioral and physiological actions of these hormones. In addition, investigating the relationships between different hormones is an important step toward understanding hormone control mechanisms. For example, plasma levels of sex steroids, from the gonads and glucocorticoids from the adrenal cortex, are generally thought to exhibit a reciprocal relationship (Greenberg and Wingfield 1987). This relationship occurs primarily because of negative interactions between the hypothalamic-pituitary-adrenal axis, responsible for glucocorticoid release, and the hypothalamic-pituitary-gonadal axis, responsible for sex steroid release (Rivier and Rivest 1991). However, other physiological factors need to be considered when trying to understand hormone control mechanisms.

One such factor, body condition, could potentially affect or be affected by steroid hormone levels. However, only a few studies in any reptile (e.g., Tokarz et al. 1998) have investigated the relationships between plasma steroid levels and body condition (mass per unit length) despite their obvious potential interactions. Corticosterone, the primary glucocorticoid in snakes (Idler 1972), can affect body condition through its role in energy mobilization. Elevated levels of corticosterone are generally observed during periods of physiological stress and act to mobilize fat stores and promote muscle catabolism and gluconeogenesis (Wingfield et al. 1998). Similarly, elevated levels of testosterone can affect body condition through suppression of the immune system and increased parasite load (Salvador et al. 1996) and increased daily activity (Marler and Moore 1988). No studies have investigated the relationship of body condition to plasma sex steroid and corticosterone levels in free-living snakes.

The purpose of this study is to describe the annual cycles of testosterone and corticosterone in the male red-spotted garter snake, *Thamnophis sirtalis concinnus*, and correlate these with breeding periods and annual variation in body condition. The common garter snake, *Thamnophis sirtalis*, is the widest ranging reptile in North America, existing from east coast to west coast and Texas into Canada. The red-spotted garter snake, *T. s. concinnus*, of the Willamette Valley of western Oregon, has an extended mating season of 10–12 wk following spring emer-

gence and can be active during 10 mo of the year (I. T. Moore, personal observation). Little is known about the reproductive endocrinology of this subspecies.

In contrast, the reproductive endocrinology of the subspecies *Thamnophis sirtalis parietalis* is one of the most investigated of any reptile (e.g., Crews 1984, 1991; Crews et al. 1984; Mason and Crews 1985; Krohmer et al. 1987; Whittier et al. 1987; Mason et al. 1989). The red-sided garter snake, *T. s. parietalis*, lives at the northern limit of the species' range and has a limited activity period of 4 mo during the spring and summer followed by 8 mo of winter dormancy. Mating occurs in the spring when testosterone levels in males are declining and testes are regressed (Krohmer et al. 1987). Testes are fully recrudesced and testosterone levels peak in the late summer before the animals enter winter dormancy (Weil 1985; Krohmer et al. 1987).

These studies have not found a relationship between plasma corticosterone levels and sex steroid levels and have not investigated cycles of body condition. Considering the wide array of environments this species inhabits, we hypothesized that population differences in reproductive cycles exist. Furthermore, we wanted to investigate the relationships between sex steroid hormones and glucocorticoid stress hormones cycles as well as associated cycles in body condition. By investigating these factors together, we hoped to elucidate their control and function in a free-living vertebrate.

Material and Methods

Sampling

Male *Thamnophis sirtalis concinnus* were captured from underneath cover boards during a 2-yr period (1995, $n = 18$; 1996, $n = 60$) at the E. E. Wilson Wildlife Area, 15 km north of Corvallis, Oregon. Animals were captured during 10 mo of the year, as no samples were obtained during December and January, when animals could not be found. Immediately after capture, each individual was bled by cardiac puncture, and a 100-μL sample of whole blood was obtained. All blood samples were obtained during the natural daily activity period of the animal to avoid potential confounding diel variation. On average, blood samples were obtained within 163 s of sighting the animal.

After taking the blood sample, snout-vent length (± 1 mm) and mass (± 1 g) were measured on each individual. Animals were individually marked and released at the original site of capture after all measurements were obtained. No animals were bled more than once during the study. Blood samples were stored on ice until return to the laboratory, where they were centrifuged. The plasma was separated using a 100-μL Hamilton syringe, and plasma samples were frozen ($-60°C$) until assayed for corticosterone and testosterone. Relative body condition for each animal was calculated as its residual value from the linear regression of mass on snout-vent length for the population (Bradshaw 1986).

Radioimmunoassay

Plasma levels of testosterone and corticosterone were measured by radioimmunoassay following the procedures of Moore (1986) with modifications. Briefly, plasma volumes of 10 μL were used in the assay. For individual recovery determination, each sample was equilibrated overnight with 2,000 cpm of tritiated testosterone and corticosterone (Amersham). Each sample was then extracted twice in 2 mL of diethyl ether, and the ether phase was removed and dried in a warm water bath under a stream of nitrogen gas. The extracts were then resuspended in 10% ethyl acetate in isooctane. The samples were chromatographed through individual celite columns to separate the steroid fractions and neutral lipids. The fractions were eluted using stepwise increasing proportions of ethyl acetate in isooctane. The purified eluates were dried and resuspended in buffer (phosphate buffered saline with 0.1% gelatin) for the assay.

For the assay, individual sample recoveries were determined from 50 μL of the sample, while 200 μL of the sample was allocated to each of two duplicates. Serial dilutions for the standard curves were performed in triplicate. All samples, including serial dilutions and 100% bounds, were incubated overnight with 100 μL of antibody (testosterone antibody T-3003 from Wein and corticosterone antibody B21-42 from Endocrine Sciences) and 100 μL of tritiated steroid. Unbound steroid was separated using dextran-coated charcoal, and the bound steroid was decanted into scintillation vials. The samples were resuspended in 4 mL of toluene-based scintillation fluid, incubated for 12 h, and counted on a Beckman LS1800 scintillation counter. A cubic spline curve was fitted to the standard curve points, and final steroid concentrations were calculated from this curve and adjusted based on individual recoveries. Intraassay variation was 14% for corticosterone and 10% for testosterone, calculated from an assay of standards ($n = 16$). Samples were analyzed in two assays, with an interassay variation of 17% for corticosterone and 12% for testosterone. Limits of detectability were approximately 1.30 ng/mL for corticosterone and 0.30 ng/mL for testosterone.

Statistics

Hormone levels across months were compared by a Kruskal-Wallis one-way ANOVA on ranks. The relationships between plasma levels of testosterone and corticosterone as well as between snout-vent length and mass were determined by linear regression analysis. Body condition across months was compared by one-way ANOVA. Post hoc multiple comparisons were done using Dunn's method. Corticosterone levels between individuals with positive versus negative body conditions were compared by the rank sums test. For all tests the level of significance was $P < 0.05$. All data were analyzed using the Jandel SigmaStat Version 2.0 statistical package (SPSS 1997).

Results

Steroid Cycles

Plasma levels of testosterone varied significantly by month (Fig. 1; Kruskal-Wallis one-way ANOVA on ranks, $H_{1,9} = 37.517$, $P < 0.001$). Testosterone displayed a peak in September, with levels that were higher than in March, May, or June, and February levels were higher than in May (Dunn's method, $P < 0.05$). Plasma levels of corticosterone also varied significantly by month (Fig. 1; Kruskal-Wallis one-way ANOVA on ranks, $H_{1,9} = 34.822$, $P < 0.001$). Levels of corticosterone were higher in September than in June or August (Dunn's method, $P < 0.05$). There was a significant positive relationship between plasma levels of the natural log transformed values of the two steroids (Fig. 2; linear regression, $r^2 = 0.10$, $P = 0.005$).

Body Condition Cycles

There was a significant relationship between mass and snout-vent length (linear regression, $r^2 = 0.86$, $P < 0.001$). Body condition, the residual of the regression of mass on snout-vent length, varied by month (Fig. 3; ANOVA, $F_{1,9} = 2.490$, $P = 0.016$). There was a significant negative relationship between plasma levels of corticosterone and body condition (linear regression, $r^2 = 0.08$, $P = 0.013$). Individuals with a negative body condition had significantly higher levels of plasma corticosterone than did individuals with a positive body condition (Fig. 4; rank sum test, $T_1 = 1,115.00$, $P = 0.005$). There was no relationship between body condition and testosterone levels.
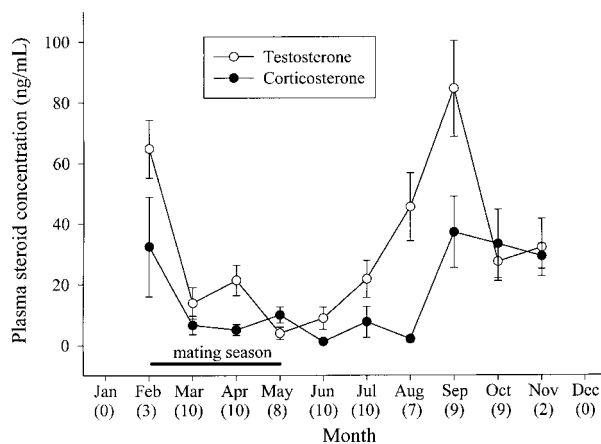

Figure 2. Relationship of ln-converted plasma testosterone and ln-converted plasma corticosterone levels.

Figure 1. Annual cycles of plasma steroid hormone levels (mean ± SE) in male red-spotted garter snakes during the 2 yr of the study. The numbers in parentheses represent the sample size for that month.
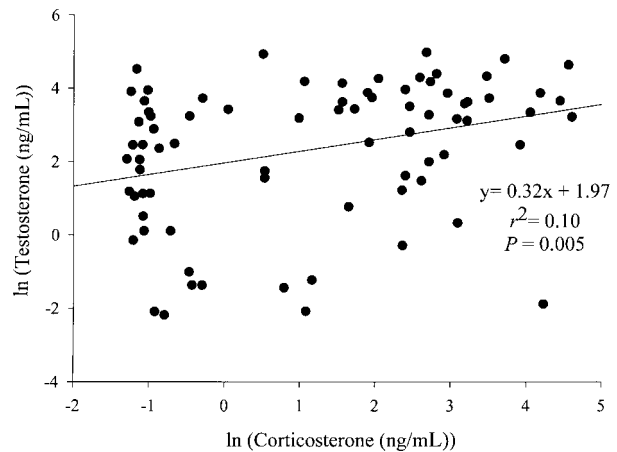
Discussion

Testosterone Cycle

As expected, male red-spotted garter snakes displayed an annual cycle of plasma testosterone with a peak in September and lowest levels during May. This coincides with previous morphological and histological evidence of testes cycles from the closely related San Francisco garter snake, *Thamnophis sirtalis infernalis*; Western terrestrial garter snake, *Thamnophis elegans terrestris*; and Pacific Coast aquatic garter snake, *Thamnophis atratus* (Fox 1952, 1954). This also appears to correlate with the estival (summer) and postnuptial pattern described by Saint Girons (1982). Interestingly, our study found testosterone levels elevated above basal levels at the beginning of the mating season (February, March, and April). Similarly, testosterone levels are elevated in *Thamnophis sirtalis parietalis* (Krohmer et al. 1987) and in *Thamnophis sirtalis sirtalis* (Weil 1985) when the snakes emerge from hibernation and then decline in the following weeks. The presence of plasma testosterone during the spring may be associated with mating behavior, while the fall peak is associated with gametogenesis and potential fall mating. Although previous studies suggest that the testes are regressed during the spring in this species (Crews et al. 1984; Krohmer et al. 1987), the presence of measurable plasma testosterone levels suggest that the testosterone-producing leydig cells may be active and steroidogenic.

Corticosterone Cycle

Corticosterone levels also varied through the year, with the highest levels were in September and the lowest in June. The levels of corticosterone we found are lower than those reported for *T. s. parietalis* in Manitoba, Canada (Krohmer et al. 1987;
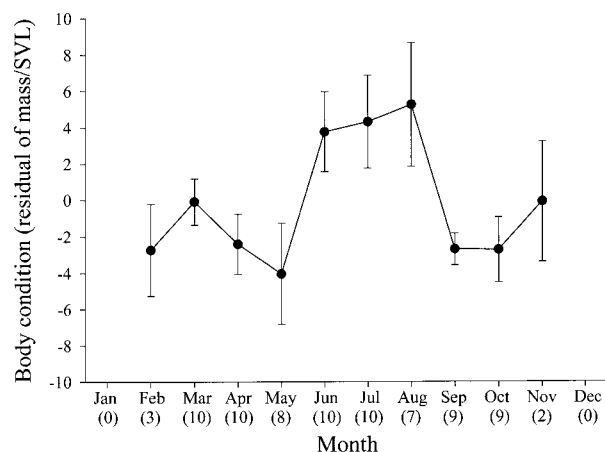
Figure 3. Annual cycle of body condition (mean ± SE) in male red-spotted garter snakes during the 2 yr of the study. Body condition is defined as the residual from the regression of mass on snout-vent length. The numbers in parentheses represent the sample size for that month.

Moore et al. 2000). The difference in plasma levels of corticosterone between subspecies may be an adaptation to unique environments. The relatively low levels of corticosterone we report here, as compared to *T. s. parietalis*, could represent less abiotic stress or milder environmental conditions experienced by *Thamnophis sirtalis concinnus*.

Corticosterone levels in *T. s. concinnus* cycle with testosterone levels throughout the year and there is a significant positive correlation between plasma levels of the two steroids when compared across all months. Although glucocorticoids in general are thought to have negative effects on sex steroids and reproduction (Greenberg and Wingfield 1987), this is not universal (Wilson and Wingfield 1994; Knapp and Moore 1997; Tyrrell and Cree 1998). Corticosterone and testosterone are positively correlated in the annual cycle of the Galápagos tortoise, *Geochelone nigra* (Schramm et al. 1999). More generally, a positive association between plasma corticosterone and reproduction was noted in the side-blotched lizard, *Uta stansburiana*, where corticosterone levels parallel annual activity patterns (Wilson and Wingfield 1994). The male crested newt, *Triturus carnifex*, displayed two peaks in corticosterone, one at the beginning of the breeding season and one as they leave the pond, that appear associated with levels of activity (Zerani and Gobbetti 1993). However, this newt displayed a significant negative relationship between corticosterone and testosterone (Zerani and Gobbetti 1993). In our study, the lack of a negative relationship between the steroids suggests that there is not a direct negative effect of corticosterone on testosterone in this subspecies of garter snake. The presence of a significant positive relationship between the two steroids could occur because the

energetic costs associated with elevated testosterone (gametogenesis in the late summer and mating in the spring) necessitate increased plasma corticosterone to mobilize energy stores. In addition, this positive association may be a mechanism that allows the animals to respond to stressors, during certain seasons, with increases in plasma corticosterone while maintaining sufficient plasma levels of testosterone to mediate sex steroid dependent processes.

Body Condition Cycle

In addition to annual cycles of testosterone and corticosterone, male *T. s. concinnus* display an annual cycle of body condition. The annual cycle suggests that individuals are significantly more robust during the summer than during the spring and fall. This cycle is surprising because we expected to see the most robust animals in the fall after a summer of feeding and in preparation for hibernation. However, the energetic costs of winter dormancy may be minimal for poikilotherms, which do not metabolically maintain body temperature as temperatures decline. Based on our results, we hypothesize that low body condition in the spring is associated with the lack of food availability and the active searching and courtship displayed by these animals in an effort to mate. During the summer there is an abundance of food, and body condition is elevated. The decline in body condition in the fall could be associated with a decline in food availability and the energetic costs of gametogenesis during this period.

Individuals with a negative body condition had significantly higher levels of corticosterone than did individuals with a positive body condition. Corticosterone has been demonstrated to play a role in energy mobilization and utilization (Wingfield et
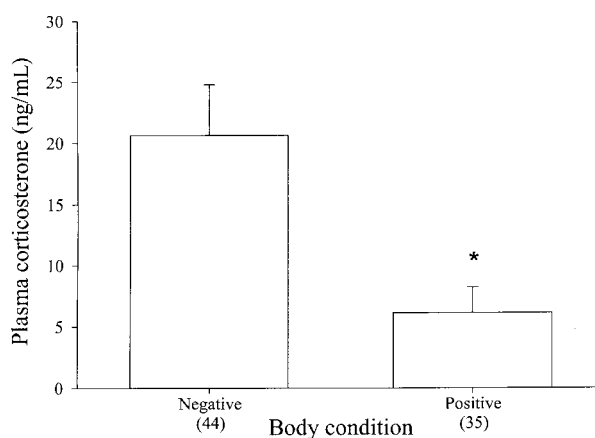

Figure 4. Plasma corticosterone levels (mean ± SE) between individuals with negative and positive relative body conditions. Body condition is defined as the residual from the regression of mass on snout-vent length (*asterisk, P < 0.05*).

al. 1995). Thus, it is not surprising that individuals of low body condition had elevated plasma corticosterone levels. The lack of an association between testosterone and body condition is interesting because some studies have argued for fitness benefits of increased testosterone (e.g., increased territory size and quality [Fox 1983]). In contrast, others have suggested that there may be fitness costs to elevated testosterone levels, such as increased parasite load (Salvador et al. 1996) and increased activity, as well as decreased survivorship (Marler and Moore 1988). Our data do not support either of these contentions; however, we did not directly test these hypotheses experimentally.

In conclusion, we describe annual cycles of testosterone, corticosterone, and body condition that are closely linked in male *T. s. concinnus*. Testosterone levels are highest in the spring and fall, when the snakes are most reproductively active. Corticosterone levels are positively associated with testosterone levels but negatively associated with body condition. Thus, corticosterone may be the linchpin that unites the two other physiological factors.

Acknowledgments

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