

The role of androstenedione and testosterone in the reproduction and antler growth of a male white-tailed deer

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Abstract. Seasonal levels of androstenedione and testosterone were investigated in plasma of mature intact and castrated male white-tailed deer. In four intact bucks, androstenedione concentrations were low in February and March (around 1 nmol/l) and then increased significantly ($P < 0.05$) from April to November (peak 2.34 nmol/l). Testosterone remained low (below 3.5 nmol/l) from February to August and then rose significantly ($P < 0.01$) till November (peak 36.78 nmol/l). Both hormones declined from November to February. In three castrates, androstenedione levels remained virtually unchanged (averaging around 0.5 nmol/l) between January and September. After a rapid significant increase ($P < 0.05$) till November (peak value 2.45 nmol/l), androstenedione concentrations declined quickly to a baseline level. Testosterone in castrates remained around 0.3 to 0.7 nmol/l for most of the year with a non-significant peak (1.45 nmol/l) in October.

These data indicate that the spring and summer increase in androstenedione in the intact deer is of testicular origin; the fall peak, however, may be a result of increased production in the adrenal glands.

It can be speculated that the increase in androstenedione in the blood of the male deer during the spring may be responsible for the seasonal initiation and support of antler growth as well as being supportive to the re-activation of the reproductive system.

White-tailed deer are seasonal breeders which exhibit a photoperiodically-controlled reproduc-

tive cycle (Bubenik 1983). Most boreal deer are short-day breeders, and therefore their reproductive system is relatively inactive during the spring. In early summer the concentration of gonadotropins in the blood increases, followed by reactivation of spermatogenesis and an increase in the levels of testosterone (T) in blood. The peak of reproductive stimulation is reached during the rutting season in the autumn (Mirarchi et al. 1977a,b, 1978; Bubenik et al. 1982). As the deer undergo yearly re-activation of the reproductive system, this process has been compared to 'annual puberty' (Lincoln 1971).

The activation of the hypothalamo-pituitary-gonadal axis during puberty is a rather complicated process which has not yet been entirely elucidated. In the early stages of human puberty, the progress of sexual maturation correlates better with adrenal function than with gonadal activity (Ducharme et al. 1976). Some authors speculated that the beginning of puberty is tied to activation of the adrenal cortex and the production of adrenal androgens. Plasma levels of adrenal androgen androstenedione (Δ_4 -A) were reported to be the best index of sexual maturation (Ducharme et al. 1976; Parker et al. 1978). As Δ_4 -A may play a role in initiation of puberty in the human, it could be hypothesized that yearly re-activation of the reproductive system in seasonal

breeders may also be connected to circannual variation of this androgen. In our recent study, we have tested this hypothesis on the sexually mature white-tailed deer.

Materials and Methods

Four sexually mature male white-tailed deer (*Odocoileus virginianus*) (ages 2, 3, 5 and 8 years) were kept in individual pens and fed an ad libitum diet of pelleted deer ration supplemented with grass, hay and browse. Three consecutive blood samples (30 min apart) were taken monthly for a full year from the jugular vein via an indwelling catheter, with the bucks being anaesthetized with xylazine (Rompun®) and ketamine (Ketaset®) mixed 1:1. In addition, in three mature bucks, (ages 3, 4 and 6 years) castrated at least 2 years before the experiment, blood was sampled like in the intact deer once a month for 1 or 2 consecutive years. However, only one blood sample was taken each time. The heparinized blood was immediately centrifuged, and plasma frozen for later Δ_4 -A assays.

RIAs for T and Δ_4 -A

Plasma concentrations of T were determined by a highly specific radioimmunoassay which was described in more detail in our previous publication (Bubenik et

al. 1982). The sensitivity of the assay is about 0.2 nmol/l. The interassay variation was about 7.5–12%. There was only negligible cross-reaction with androstanolone and androstenedione.

For assay of Δ_4 -A plasma was extracted twice with diethyl ether. The extracts were pooled, dried and resuspended in assay buffer. Typical recovery of androstenedione in this procedure is greater than 90%, hence correction of individual samples for procedural losses was not monitored and the data are presented as non-corrected values. The preparation of the rabbit antiserum against androstenedione has been described and cross-reactivity with testosterone found to be 2%, with 5 α -androstenedione (2, (5 α)-androsten-3-ol-17-one) 5%, and with all other steroids tested less than 1% (Leung & Armstrong 1979). The assay procedure was that reported by Jansz & Pomerantz (1984). The sensitivity of the assay was less than 20 pmol/assay tube and the interassay coefficient of variation less than 11%. All the samples in each assay have been analyzed in the same series.

Statistics

The data were subjected to a one-way analysis of variance. When F values indicated significance, differences between means were compared by a multiple range test (Duncan 1955). Because of small numbers of samples, the first- and second-year data of castrates were combined into one year seasonal profile.

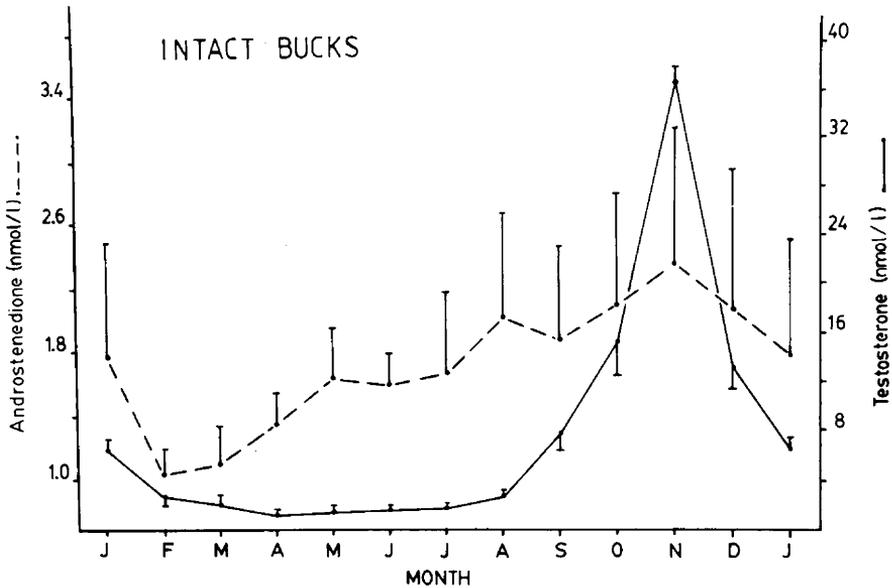


Fig. 1.

Seasonal levels of testosterone and androstenedione in plasma of four intact adult male white-tailed deer. Each point on the graph represents 4 individual animals. The average value of each buck was calculated from the mean of levels detected in 3 samples taken 30 min apart. Vertical bars indicate standard error of mean values.

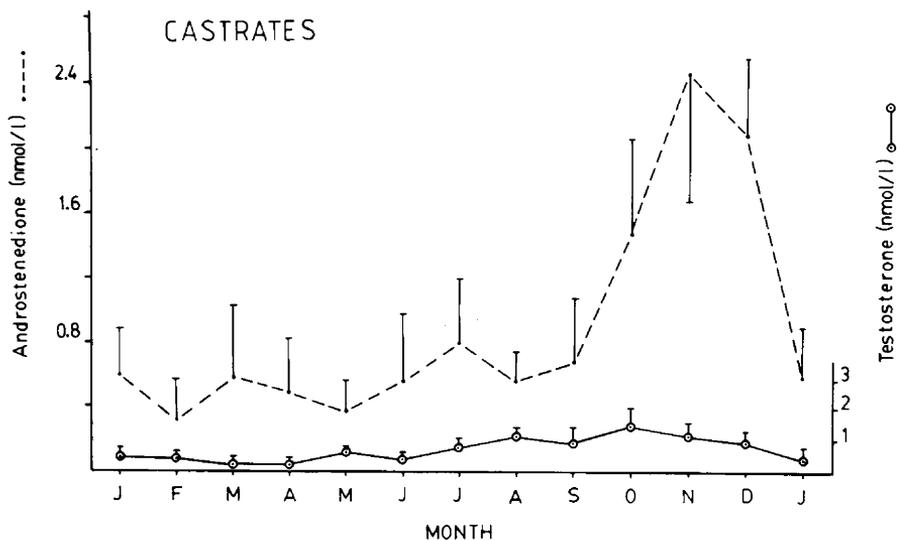


Fig. 2.

Seasonal levels of testosterone and androstenedione in castrated deer. Each point on the graph represents the mean of 4–6 samples.

Results

Intact bucks

T levels exhibited variations typical for male white-tailed deer (Fig. 1). Low values (1 to 2.8 nmol/l) were registered between February and August. A rapid increase in T levels from August to November (peak value 36.78 nmol/l) was followed by a quick decline till February. Statistically, peak levels of T in November are higher than in any other month ($P < 0.01$). Concentrations of T in September, October, December and January are higher than in the rest of the year ($P < 0.05$), except in November.

Androstenedione levels were low (around 1 nmol/l) during February and March. Then they began a steady, but not particularly steep increase reaching peak values (2.34 nmol/l) in November. A slow decrease between November and January was followed by a much faster decline until February. Levels of Δ_4 -A in June were significantly higher ($P < 0.05$) than the concentrations in February.

Castrates

In the majority of samples, T values were only slightly above detection level between January and

June. A very moderate increase till October (peak value 1.45 nmol/l) was followed by a steady, slow decline till January (Fig. 2). No statistically significant differences in seasonal variation of T was detected.

Androstenedione levels were rather stable between January and September (around 0.5 nmol/l). A sharp increase between September and November (peak value 2.45 nmol/l) was followed by a fast decline toward January (Fig. 2). Statistically, mean values of Δ_4 -A in November and December were higher ($P < 0.05$) than the concentrations in February, March and April.

Androstenedione of intact vs castrated bucks

The mean values of Δ_4 -A in intact bucks were significantly higher ($P < 0.05$) in May, July and August than the comparative concentrations in castrates (Fig. 3).

The large standard error of Δ_4 -A is probably due to major variations of this hormone in plasma. Differences up to 40% between samples taken 10 min apart were detected (Bubenik, Smith and Pomerantz – unpublished observations).

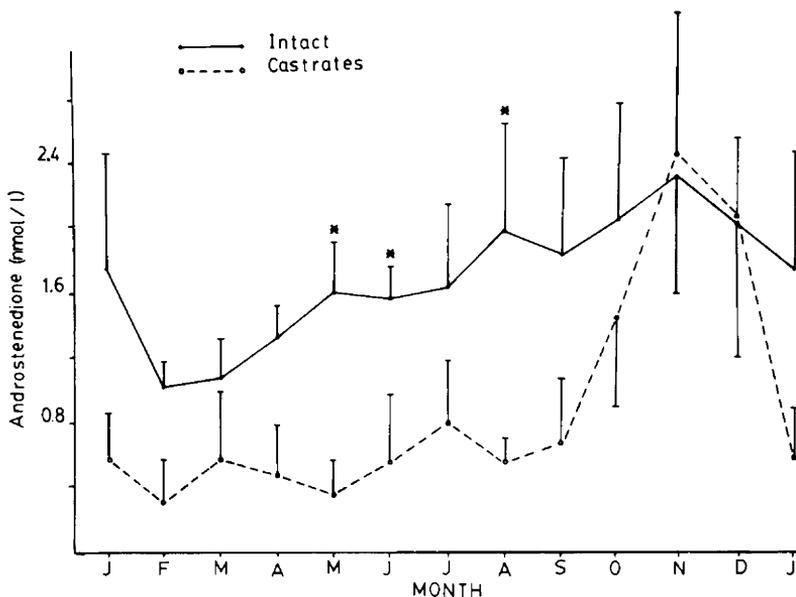


Fig. 3.

Comparison of seasonal levels of androstenedione in intact and castrated bucks. * Indicate significantly higher levels ($P < 0.05$) of intact bucks as compare with castrates.

Discussion

Dehydroepiandrosterone and androstenedione are adrenal androgens reported to be most closely correlated with human puberty (Ducharme et al. 1976; Parker et al. 1978; Ducharme 1981). In addition to synthesis in the adrenal cortex, Δ_4 -A is also produced in the testes. In most mammals, including ruminants, Δ_4 -A is the predominant testicular androgen before sexual maturity (Rawlings et al. 1972). Δ_4 -A is a main precursor of T (Brooks 1975) and T can also be converted back to Δ_4 -A (Martini 1982). As both the adrenal glands and the testes are capable of producing Δ_4 -A, it is difficult to distinguish the source of this hormone during the seasonal cycle of the intact male deer.

Except for a very short period in the spring, the levels of LH in male white-tailed deer do not increase significantly until June (Bubenik et al. 1982) and the testicular activity does not change much till May (Mirarchi et al. 1977b). Data from intact bucks only may lead to speculation that the spring increase in Δ_4 -A is due to activation of the adrenal cortex. However, results from castrated

deer point to another explanation. First, peak values of Δ_4 -A in castrates and intact bucks in the autumn are found in the identical month (November) and are in the same range (2.34 nmol/l vs 2.45 nmol/l). Secondly, a spring increase in Δ_4 -A found in the intact deer, was not observed in castrates (Fig. 3). As it can be assumed that in castrates most of the Δ_4 -A is of adrenal origin, the seasonal differences between intact and orchietomized bucks may reflect the production of Δ_4 -A in the testes. In that case less than half of the basal levels of Δ_4 -A is of adrenal origin; the remaining portion is secreted by the testes. The dramatic increase in Δ_4 -A observed in castrates around the rut but not found in intact bucks would indicate that whereas the adrenal production of Δ_4 -A is stepped up at that time, the testicular production of androgens is shifted toward T. A similar switch in androgen production was found in other gonadal cells (Erickson et al. 1985).

The elevation of Δ_4 -A in intact bucks in April may be related to a short-term spring activation of

deer testes (Bubenik et al. 1982). This activation may be characterized in white-tailed deer by only a transient increase in T (Bubenik et al. 1982), but by a steady increment of Δ_4 -A. It was proposed by Lincoln (1971) that seasonal breeders such as the deer are undergoing a seasonal 'annual puberty'. It can be hypothesized that this repeated puberty is characterized by an increase in Δ_4 -A similar to that observed in continuous breeders during their initial puberty. Whereas in early studies, seasonal levels of T and Δ_4 -A in testes of red deer (Lincoln 1971) and plasma of white-tailed deer (Mazur 1973) were found to run virtually parallel, more recently studies report substantial differences (Brown et al. 1983a). Data obtained in fawns support the hypothesis associating Δ_4 -A with puberty. The first increase in androgen levels in male fawns which is causing the growth of first antlers (it may be called the 'initial puberty') occurs in the fall. The 'annual puberty' of adult deer begins in the spring. However, both activation of the reproductive system (the initial one in fawns as well as the annual in adults) coincides with an increase in Δ_4 -A levels (Brown et al. 1983a,b).

In several studies, the seasonal re-activation of the gonads in male deer (as measured by the volume of testes) was found to be slightly ahead of the increase in T in plasma (Lambiase et al. 1972; Mirarchi et al. 1977b). In view of the results of our present study, it may be hypothesized that it is another androgen than T (perhaps Δ_4 -A) which is first produced in the deer testes after the period of winter quiescence.

The present study showed that the adrenal glands of white-tailed deer are capable of producing an amount of T equal to or exceeding the minimal levels found in intact bucks in April (Fig. 1). Such a remarkable capacity of white-tailed deer adrenals to secrete T and Δ_4 -A may explain why in this species the growth of antlers in castrates is well under control. In the roe-deer, on the contrary, most castrates will perish within two years after gonadectomy as a result of tumour-like proliferation of their antlers (Bubenik 1963).

The seasonal levels of Δ_4 -A in plasma seems to correlate well with the seasonal re-activation of the antler growth. Therefore, Δ_4 -A might be the hypothetical, long-thought 'antler growth stimulus' predicted by Wislocki (1943) which should be responsible for initiation and maintenance of antler growth in deer.

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