Effect of oral melatonin administration on sex hormone, prolactin, and thyroid hormone concentrations in adult dogs

Patricia F. Ashley, DVM, DACVD; Linda A. Frank, MS, DVM, DACVD; Lynn P. Schmeitzel, DVM, DACVD; Elizabeth M. Bailey, MS; Jack W. Oliver, DVM, PhD

Objective—To determine the effect of oral melatonin (MT) administration on serum concentrations of sex hormones, prolactin, and thyroid hormones in dogs.

Design—Prospective study.

Animals—8 male and 8 female adult sexually intact dogs.

Procedure—5 male and 5 female dogs were treated with MT (1.0 to 1.3 mg/kg [0.45 to 0.59 mg/kg] of body weight, PO, every 12 hours for 28 days; the other 6 dogs were used as controls. Blood samples were collected on days 0, 14, and 28, and serum concentrations of estradiol-17β, progesterone, testosterone, androstenedione, estrone, androstenedione, 17-hydroxyprogesterone (17-HP), dihydroepiandrosterone sulfate (DHEAS), prolactin, and thyroxine were determined. On day 5, serum MT concentrations were measured before and periodically for up to 8 hours after MT administration in 4 treated dogs.

Results—Female dogs treated with MT had significant decreases in serum estradiol, testosterone, and DHEAS concentrations between days 0 and 28. Male dogs treated with MT had significant decreases in serum estradiol and 17-HP concentrations between days 0 and 28. Serum MT concentrations increased significantly after MT administration and remained high for at least 8 hours. Prolactin and thyroxine concentrations were unaffected by treatment.

Conclusions and Clinical Relevance—Melatonin is well absorbed following oral administration and may alter serum sex hormone concentrations. (J Am Vet Med Assoc 1999;215:1111–1115)

Melatonin (MT) is an indoleamine hormone secreted by the pineal gland in response to low light intensity and decreasing duration of daylight. It plays a role in photoperiod-related functions, such as sleep, coat development, thermoregulation, and behavior, and influences reproductive development and function in several species, particularly species with seasonal breeding patterns. Pharmacologic doses of MT have been used to manipulate the reproductive cycles of animals. For instance, Suffolk rams typically breed during the time of year when days are short, but administration of MT implants during periods when days are long will cause a premature increase in breeding activity. Melatonin can also affect reproductive cycles in mammals less affected by season. Daily long-term administration of MT to women inhibits ovulation by reducing luteinizing hormone (LH), progesterone, and estradiol concentrations. These hormonal effects are believed to be mediated by a negative influence on secretion of hypothalamic gonadotropin releasing hormone (GnRH) or by a direct, negative effect on LH secretion from the pituitary gland.

Melatonin influences hair growth in species with seasonal coat changes. Subcutaneous implantation of MT during the summer results in premature growth of thick winter fur in mink, which is associated with a decrease in prolactin concentrations. Inhibition of prolactin secretion alone by the prolactin inhibitor bromocriptine also induced early growth of winter fur, suggesting that the effect of MT on hair growth may be mediated through its effect on prolactin secretion. Alternatively, MT may have a direct effect on the hair follicle. Hair follicles from Cashmere goats cultured in vitro with MT grew significantly longer hair shafts than did follicles cultured without MT.

Canine recurrent flank alopecia (CRFA), or seasonal flank alopecia, is a condition of unknown cause that is speculated to result from a relative deficiency of MT or an abnormal response of the skin to fluctuations in photoperiod. Dogs with seasonal flank alopecia have unilateral or bilateral alopecia of the thoracolumbar region that usually starts when duration of daylight is decreasing. Many, but not all, dogs regrow hair within 3 to 6 months, and 50% of affected dogs will have a recurrence of alopecia the next fall. In a study by Paradis, 9 dogs with recurrent seasonal flank alopecia were given MT by injection or implantation, and none of the dogs developed alopecia the following treatment.

How MT affects hair growth in dogs with CRFA is unknown. Melatonin may have a direct effect on the hair follicle, or as in mink, MT may act indirectly by altering concentrations of other endogenous hormones that affect hair growth. Sex hormones and thyroxine (T4) are known to influence hair growth. In the study by Paradis, serum MT concentrations and concentrations of other endogenous hormones were not evaluated. The purpose of the study reported here was to determine the effect of daily oral administration of MT on serum concentrations of sex hormones, prolactin, and T4 in dogs. Serum MT concentrations after administration of MT were also measured.
Materials and Methods

Dogs—Sixteen adult sexually intact (8 male and 8 female) dogs were used in the study. Dogs were housed and maintained according to the university animal care and use committee guidelines. The light-dark cycle was maintained at 12 hours of light and 12 hours of darkness. A physical examination, CBC, serum biochemical analyses, urinalysis, heartworm test, and fecal flotation test were performed on all dogs prior to the study. In addition, vaginal smears from the female dogs were examined cytologically prior to the study. Physical examinations and cytologic examination of vaginal smears were repeated weekly throughout the study.

Experimental protocol—Ten dogs (5 males and 5 females; mean body weight, 22.9 kg [50.3 lb]; range, 19.0 to 27.7 kg [40 to 61 lb]) were randomly assigned to the MT treatment group. Dogs in this group were treated with 25 mg of MT (1.0 to 1.3 mg/kg [0.45 to 0.59 mg/lb] of body weight), PO, every 12 hours. Melatonin was administered by mixing it with lactose powder and placing it in a gelatin capsule. The remaining 6 dogs (3 males and 3 females; mean body weight, 17.9 kg [39.4 lb]; range, 11.4 to 27.3 kg [25 to 60 lb]) were assigned to the control group. Control dogs were given an empty gelatin capsule, PO, every 12 hours.

Blood samples were collected from all dogs on days 0, 14, and 28, and serum sex hormone, T₄, and prolactin concentrations were measured. Serum MT concentrations were determined on day 5 in 4 of the treatment group dogs. Blood samples were collected before (baseline) and 15, 30, 60, and 120 minutes and 4 and 8 hours after administration of MT. Research has indicated that the elimination half-life of MT in dogs is 5 hours; therefore, serum MT concentrations should have reached steady-state concentrations of MT by day 5.

All blood samples were centrifuged and serum was obtained within 90 minutes after blood sample collection. Serum samples were stored at -70°C until assays were performed. All assays, except the assay to determine prolactin concentration, were performed at the Clinical Endocrinology Laboratory at the College of Veterinary Medicine of the University of Tennessee. Prolactin concentrations were measured in the Department of Animal Science at the University of Tennessee. All tests were run in duplicate.

Sex hormone concentrations—Serum estradiol-17β, progesterone, testosterone, dehydroepiandrosterone sulfate [DHEAS], androstenedione, and 17-hydroxyprogesterone [17-HP]) concentrations were determined by use of a commercial kit. Serum prolactin concentration was determined by use of a double-antibody radioimmunoassay.

Statistical analyses—Mean serum hormone concentrations at each sampling time were compared by use of one-way repeated measures ANOVA. All pair-wise multiple comparisons were examined by use of the Student-Newman-Keuls method. To evaluate the effect of MT administration on sex hormone and prolactin concentrations, the treatment and control groups were divided into females and males for some sex comparisons, and each dog was used as its own control over time. The control group was included to ensure that significant changes over time were not attributable to external influences. Female dogs with serum sex hormone concentrations reflective of diestrus or metestrous (eg, high progesterone, 17-HP and androstenedione, and DHEAS concentrations) were not included in statistical analyses. Values of P > 0.05 were considered significant.

Results

None of the dogs had clinical signs of recent estrus at the beginning of or during the study. However, 3 dogs in the treatment group had high serum progesterone, 17-HP, and androstenedione concentrations on day 0, and 2 of these dogs had high serum DHEAS concentrations indicating that they were in diestru.

Therefore, statistical analyses of androstenedione, 17-HP, and progesterone concentrations could not be done. Data from the 2 dogs with high DHEAS concentrations were not included in statistical analyses.

Sex hormone concentrations—Serum estradiol-17β concentration was significantly decreased between days 0 and 14 and between days 0 and 28 in female and male dogs treated with MT (P = 0.001 and 0.003, respectively). Serum testosterone concentration was not significantly different between MT treatment days 0, 14, and 28.

Table 1—Serum estradiol-17β, testosterone, dehydroepiandrosterone sulfate [DHEAS], and 17-hydroxyprogesterone [17-HP] concentrations in adult, sexually intact female dogs treated with melatonin (treatment group; n = 5) and in control dogs (n = 3)

<table>
<thead>
<tr>
<th>Sex hormone</th>
<th>Treatment group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 Day 14 Day 28</td>
<td>Day 0 Day 14 Day 28</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>68.1±5.0 52.0±6.8 50.3±5.2</td>
<td>54.2±9.1 35.1±1.5 41.9±4.7</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>8.5±0.1 3.4±0.5 1.7±0.2</td>
<td>0.2±0.0 0.2±0.0 0.2±0.0</td>
</tr>
<tr>
<td>DHEAS (ng/ml)</td>
<td>2.4±0.5 0.9±0.4 0.2±0.0</td>
<td>0.2±0.1 0.2±0.1 0.2±0.1</td>
</tr>
<tr>
<td>17-HP (ng/ml)</td>
<td>0.3±0.2 0.1±0.0 0.1±0.0</td>
<td>0.2±0.1 0.2±0.1 0.2±0.1</td>
</tr>
</tbody>
</table>

n = 2: statistical analyses were not performed.

Data are given as mean ± SEM; dogs in the treatment group were given melatonin at a dosage of 1.0 to 1.3 mg/kg (0.45 to 0.59 mg/lb), PO, every 12 hours. In each row, values with different superscripts are significantly different (P < 0.05).

Table 2—Serum estradiol-17β and 17-HP concentrations in adult, sexually intact male dogs treated with melatonin (treatment group; n = 5) and in control dogs (n = 3)

<table>
<thead>
<tr>
<th>Sex hormone</th>
<th>Treatment group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 Day 14 Day 28</td>
<td>Day 0 Day 14 Day 28</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>57.2±2.8 40.4±1.5 34.1±1.0</td>
<td>44.8±8.1 23.0±7.7 28.8±4.5</td>
</tr>
<tr>
<td>17-HP (ng/ml)</td>
<td>0.4±0.1 0.2±0.1 0.2±0.1</td>
<td>0.2±0.1 0.1±0.0 0.1±0.0</td>
</tr>
</tbody>
</table>

Data are given as mean ± SEM; dogs in the treatment group were given melatonin at a dosage of 1.0 to 1.3 mg/kg (0.45 to 0.59 mg/lb), PO, every 12 hours. In each row, values with different superscripts are significantly different (P < 0.05).

Discussion

Serum sex hormone concentrations were significantly decreased in dogs treated with melatonin.
respectively), whereas serum estradiol concentration was not significantly changed over time in control dogs (Tables 1 and 2). For male and female dogs, there were marked differences between treatment and control groups in regard to day-0 serum estradiol-17β concentration (day-0 values for treatment and control groups were not compared statistically).

Serum testosterone concentration significantly \((P = 0.001)\) decreased between days 0 and 14 and between days 0 and 28 in male dogs treated with MT (Table 1); conversely, serum testosterone concentration did not significantly change over time in male dogs treated with MT or in male control dogs. In female dogs, there was a marked difference between treatment and control groups in regard to day-0 serum testosterone concentration.

Serum DHEAS concentration significantly \((P < 0.001)\) decreased between days 0 and 14 and between days 0 and 28 in female dogs treated with MT (Table 1); conversely, serum DHEAS concentration did not significantly change over time in male dogs treated with MT or in control dogs.

Serum 17-HP concentration significantly \((P = 0.001)\) decreased between days 0 and 14, and between days 0 and 28 in male dogs treated with MT (Table 2). In male dogs, there was a marked difference between treatment and control groups in regard to day-0 serum 17-HP concentration. Serum 17-HP concentration did not significantly change over time in control dogs; data for female dogs treated with MT were not analyzed, because serum 17-HP concentration was measured in only 2 dogs.

Significant changes were not found over time in serum progesterone or androstenedione concentrations in any of the groups.

Prolactin and T₄ concentrations—Mean ± SEM serum prolactin concentration on day 0 was \(2.91 ± 0.31\) ng/ml for female dogs and \(2.83 ± 0.46\) ng/ml for male dogs. Serum prolactin concentration did not change significantly over time in any of the groups. Mean ± SEM serum T₄ concentration on day 0 was \(14.9 ± 2.25\) ng/ml, and concentration did not change significantly over time in the treatment or control group.

Melatonin concentration—Serum MT concentration had significantly \((P = 0.005)\) increased, compared with baseline concentration \((mean ± SEM, 137 ± 41\) pg/ml), 15 minutes after MT administration, and it was still significantly increased 8 hours later. Between 15 minutes and 4 hours after MT administration, all serum MT concentrations exceeded the upper detection limit of the assay \((300\) pg/ml). For 3 of the 4 dogs, attempts to determine serum MT concentration by assaying multiple dilutions of the serum samples were unsuccessful; for the remaining dog, extrapolated MT concentrations were \(391, 624, 524,\) and \(311\) pg/ml 30, 60, and 120 minutes and 4 hours, respectively, after MT administration. Mean MT concentration 8 hours after MT administration was \(216 ± 32\) pg/ml.

**Discussion**

In the study reported here, daily oral administration of MT was associated with significant decreases in serum estradiol, testosterone, and DHEAS concentrations in female dogs and in serum estradiol and 17-HP concentrations in male dogs. Serum concentration of androstenedione could not be evaluated in female dogs because of low numbers of dogs for analysis. Results of previous studies suggest that MT affects serum sex hormone concentrations by enhancing or inhibiting the negative feedback loop between sex hormone concentrations in the circulation and GnRH secretion (Fig 1). In these studies, MT stimulated or inhibited GnRH secretion, depending on whether the reproductive cycles of the study subjects were affected by photoperiod. For example, in animals that typically breed when days are short (i.e. when serum MT concentrations are typically increasing), administration of MT enhanced GnRH release, causing increased secretion of LH and follicle-stimulating hormone and subsequent sex hormone production. In people and animals that typically breed when days are long, administration of MT inhibited GnRH release, causing decreased secretion of LH and follicle-stimulating hormone and, ultimately, decreased serum sex hormone concentrations. Results of studies on whether estrus in domestic dogs is affected by photoperiod are conflicting, but season and duration of daylight did not significantly influence incidence of estrus or the interestrus interval.

If MT administration decreased serum sex hormone concentrations in the dogs in the study reported here by exerting a negative effect on GnRH release, then we would have expected serum progesterone concentration to also have been decreased, because progesterone is a precursor in the steroidogenesis pathway. In the study reported here, however, serum progesterone concentration could not be statistically evaluated in female dogs because of low numbers of dogs, and it was unchanged in male dogs treated with MT. It is possible that a true effect of MT administration on serum progesterone concentration was missed, because of the small number of treated female dogs in the study.

![Figure 1](https://example.com/figure1.png)
respectively), whereas serum estradiol concentration was not significantly changed over time in control dogs (Tables 1 and 2). For male and female dogs, there were marked differences between treatment and control groups in regard to day-0 serum estradiol-17β concentration (day-0 values for treatment and control groups were not compared statistically).

Serum testosterone concentration significantly (P = 0.001) decreased between days 0 and 14 and between days 0 and 28 in female dogs treated with MT (Table 1); conversely, serum testosterone concentration did not significantly change over time in male dogs treated with MT or in male control dogs. In female dogs, there was a marked difference between treatment and control groups in regard to day-0 serum testosterone concentration.

Serum DHEAS concentration significantly (P < 0.001) decreased between days 0 and 14 and between days 0 and 28 in female dogs treated with MT (Table 1); conversely, serum DHEAS concentration did not significantly change over time in male dogs treated with MT or in control dogs.

Serum 17-HP concentration significantly (P = 0.01) decreased between days 0 and 14, and between days 0 and 28 in male dogs treated with MT (Table 2). In male dogs, there was a marked difference between treatment and control groups in regard to day-0 serum 17-HP concentration. Serum 17-HP concentration did not significantly change over time in control dogs; data for female dogs treated with MT were not analyzed, because serum 17-HP concentration was measured in only 2 dogs.

Significant changes were not found over time in serum progesterone or androstenedione concentrations in any of the groups.

Prolactin and T4 concentrations—Mean ± SEM serum prolactin concentration on day 0 was 2.91 ± 0.31 ng/ml for female dogs and 2.53 ± 0.46 ng/ml for male dogs. Serum prolactin concentration did not change significantly over time in any of the groups. Mean ± SEM serum T4 concentration on day 0 was 14.9 ± 2.25 ng/ml, and concentration did not change significantly over time in the treatment or control group.

Melatonin concentration—Serum MT concentration had significantly (P = 0.005) increased, compared with baseline concentration (mean ± SEM, 137 ± 41 pg/ml), 15 minutes after MT administration, and it was still significantly increased 8 hours later. Between 15 minutes and 4 hours after MT administration, all serum MT concentrations exceeded the upper detection limit of the assay (300 pg/ml). For 3 of the 4 dogs, attempts to determine serum MT concentration by assaying multiple dilutions of the serum samples were unsuccessful; for the remaining dog, extrapolated MT concentrations were 591, 624, 624, and 311 pg/ml 30, 60, and 120 minutes and 4 hours, respectively, after MT administration. Mean MT concentration 8 hours after MT administration was 216 ± 32 pg/ml.

Discussion

In the study reported here, daily oral administration of MT was associated with significant decreases in serum estradiol, testosterone, and DHEAS concentra-

Figure 1—Proposed mechanisms for the effects of exogenous melatonin (MT) in mammals. A) When exogenous MT is not administered, sex hormones in the circulation exert negative feedback on secretion of gonadotropin-releasing hormone (GnRH) by the pituitary (P). B) In animals that typically breed when days are long, administration of MT may enhance this negative-feedback loop, inhibiting GnRH release, which causes decreased secretion of LH and FSH and, ultimately, decreased production of sex hormones by the adrenal glands and gonadal tissues (AG). C) In animals that typically breed when days are short, administration of MT may inhibit this negative-feedback loop, enhancing GnRH release, which causes increased secretion of LH and FSH and increased production of sex hormones.

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(n = 2) in which serum progesterone concentration was measured. Alternatively, the effect of MT administration on serum sex hormone concentrations may not be at the level of the hypothalamus, but instead at gonadal or adrenal sites of hormone production or at sites where progesterone is converted to androgens and estradiol; in which case, MT administration would have minimal effects on progesterone concentrations. The significant decrease in serum DHEAS concentration among female dogs treated with MT, but not control female dogs, lends support for the hypothesis that MT affected sex hormone conversion.

In our study, day-0 estradiol-17β and testosterone concentrations for female dogs in the treatment group were notably higher than concentrations for male dogs in the control group, and day-0 estradiol-17β and 17-HP concentrations for male dogs in the treatment group were significantly higher than concentrations for male dogs in the control group. The reason for these differences is unknown, but they are likely attributable to the fluid nature of sex hormone concentrations in dogs. Other researchers have found marked fluctuations in estradiol concentrations within the same animal at different sampling times on the same day. Thus, changes in serum sex hormone concentrations found in our study may not have solely been a result of MT administration but may have reflected normal fluctuations in serum concentrations, and further research evaluating change in sex hormone concentration in a larger population of dogs is needed.

Serum prolactin concentration did not change significantly over time in any of the groups in our study. This was surprising in light of results of studies involving mink, racoons, and deer, in which prolactin concentration was consistently and significantly decreased in association with MT administration. It is possible that decreases in serum prolactin concentrations may have been seen if our study had been carried out for a longer time. Some studies in mink, for instance, did not show a decrease in serum prolactin concentrations until 6 weeks after MT implantation.

Alternatively, it may be that in dogs, effects of MT administration on prolactin secretion are determined by season. Daily MT administration to wolves had a negative effect on prolactin concentration when MT was given around the time of the summer solstice, but did not have any effect when MT was given in the fall, when endogenous prolactin concentration is naturally decreasing and endogenous MT concentration is naturally increasing. The study reported here was performed in early fall, after the summer solstice. Additional study is needed to determine whether MT administration has an effect on prolactin concentrations at different times of the year or after a longer treatment time.

Serum T4 concentration did not change significantly over time in dogs given MT in our study. Therefore, it is unlikely that MT exerts its effects via alterations in thyroid homeostasis.

Measurement of serum MT concentrations multiple times after MT administration revealed a significant, persistent rise in MT concentration, indicating good absorption of the drug. In our study, as in a previous study, high serum MT concentrations were maintained for > 8 hours after administration of a pharmacologic dose of MT. However, in dogs in our study, serum MT concentrations measured on day 5 were decreasing by 8 hours after MT administration. The concentration of MT and the pattern of MT concentration (pulsatile vs constant) needed for any clinical effect is unknown. However, if constant high concentrations of MT are needed, administration every 8 hours would be necessary. If changes in serum sex hormone concentrations found in our study were associated with MT concentration, constant high MT concentrations are probably not necessary; at least in regard to alterations in serum hormone concentrations.

We elected to administer MT orally in the study reported here, because oral formulations of MT are more readily available to veterinarians and clients in the United States than are MT implants. A commercial, purified MT formulation was chosen rather than an over-the-counter MT supplement to ensure accurate dosing. The dosage of MT chosen for our study was greater than that recommended by many veterinary dermatologists for treatment of CRFA in dogs and the dosage used by Paradis, but was similar to the dosage used in people and mink, which has effects on serum prolactin and sex hormone concentrations. A high daily dose of MT is needed in people to alter sex hormone concentrations.

The mechanism by which MT stimulates or prevents hair loss in dogs with CRFA and other alopecic disorders is unknown. The study reported here provides evidence that MT may alter synthesis of sex hormones, which are known to influence hair growth.

MT may have a beneficial role in other alopecic disorders in dogs, such as adrenal hyperplasia-like syndrome, which is caused by a partial deficiency of 1 enzyme in the pathway of steroid synthesis in the adrenal gland, causing increases in some adrenal sex hormones. Further studies investigating the effects of MT implants and lower doses of MT, as well as the effects of MT administration on serum sex hormone concentrations in neutered animals, may give further insight into the role that MT and these alterations play in canine alopecic disorders.

Melaconin. Sigma Chemical Co, St Louis, Mo.
Estradiol 17β- [113] RIA, ICN Pharmaceuticals Inc, Costa Mesa, Calif.
Progesterone [113] RIA, Diagnostic Products Corp, Los Angeles, Calif.
Testosterone [113] RIA, Diagnostic Products Corp, Los Angeles, Calif.
DHEAS [113] RIA, ICN Pharmaceuticals Inc, Costa Mesa, Calif.
17-OHP [113] RIA, ICN Pharmaceuticals Inc, Costa Mesa, Calif.
Androstenedione [113] RIA, ICN Pharmaceuticals Inc, Costa Mesa, Calif.
Diagnostic Products Corp, Los Angeles, Calif.
Dr A. F. Parlow, Pauley Hormones and Antiserus Center, Harbor-UCLA Medical Center, Torrance, Calif.
ICN Pharmaceuticals Inc, Costa Mesa, Calif.

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References


5. Webster JR, Barrell GK. Advancement of reproductivity activity, seasonal reduction in melatonin secretion and seasonal pelage changes in puberal red deer hinds (Cervus elaphus) subjected to artificially shortened daily photoperiod or daily melatonin treatments. J Reprod Fertil 1985;73:235–266.


