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Plasma Thyroxine and Cortisol under Basal Conditions and during Cold Stress in the Aging Dog[1] (42549)

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Abstract. The effects of aging on plasma concentrations of thyroxine (T_4) and cortisol and on responses of these hormones to low ambient temperatures were determined in the dog. Female beagle dogs were divided into three age groups: old, adult, and puppies. The mean (±SD) ages were 11.4 ± 0.2 years, 3.0 ± 0.4 years, and 7.6 ± 0.2 weeks, respectively. All dogs came from a genetically homogeneous colony and were free from any disease. The adult and old dogs were used during anestrus. Based on four daily blood samples, the mean (±SE) T_4 level in the old dogs (2.8 ± 0.1 μg/dl) was significantly ($P < 0.001$) lower than that in the adults (4.2 ± 0.2 μg/dl) and puppies (4.4 ± 0.2 μg/dl). By contrast, mean plasma cortisol levels in the old dogs (21.1 ± 3.1 ng/ml) and adults (15.4 ± 2.4 ng/ml) were significantly higher than those in the puppies (7.2 ± 1.1 ng/ml). No significant changes in plasma T_4 and cortisol occurred in any of the three age groups at 22°C or during exposure to 10 or 4°C. Exposure to −5°C, however, produced significant increases in T_4 (>130% by 5 hr) and cortisol (>280% by 1 hr) in adult dogs. This temperature produced only a modest increase in T_4 (70% by 3.5 hr) and no change in cortisol in the old dogs. The puppies showed no change in T_4 and cortisol during exposure to −5°C. The results demonstrate that with advancing age, plasma T_4 and cortisol concentrations change in opposite directions, thus supporting the hypothesis of a negative relationship between these two hormones. These results also show that the responses of these hormones to the stress of cold decline during aging and are not yet developed in the very young.

Over the years, several investigators have made the interesting speculation that a reciprocal relationship exists between the secretion of glucocorticoids and thyroid hormones. Thus, experimental increases in circulating levels of glucocorticoids have been shown to inhibit secretion of thyroid hormones (1–3), suppress plasma levels of thyroid-stimulating hormone (TSH), decrease TSH response to thyrotropin-releasing hormone (TRH), and impair TRH secretion in several species (4–6). However, a negative relationship between adrenal and thyroid activities under natural conditions has not been demonstrated.

The present study was conducted to investigate changes in plasma levels of thyroxine (T_4) and cortisol in the aging dog and to explore the possibility that these changes, if any, would be in opposite directions. These hormones have rarely been determined simultaneously in aging animals, and when they have been measured individually, the results have often been conflicting. Thus, increases, decreases, or no changes in circulating levels of T_4 (7–14) and glucocorticoids (15–20) have been reported in various species. The reasons for these discrepancies are not clear but may be related to several uncontrolled variables including the stages of nutrition and health and the use of an inadequate number of age groups (17). In this study, we avoided a number of these variables and further investigated the effects of aging on T_4 and cortisol release by determining the responses of these hormones to the stress of low ambient temperatures in animals of three age groups.

Materials and Methods. Fifteen female beagle dogs were divided into three age groups of 5 each: old, adult, and puppies. The mean (±SD) ages were 11.4 ± 0.2 years, 3.0 ± 0.4 years, and 7.6 ± 0.2 weeks, respectively. The old dogs weighed (mean ± SD) 9.4 ± 0.9 kg, the adults 10.7 ± 1.3 kg, and the puppies 2.4 ± 0.4 kg. All dogs were housed individually in aluminum cages in an air-conditioned (22 ± 2°C), light-controlled (lights on from 0700

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to 1900 hr) room. They were fed dog chow and provided water *ad libitum*.

The effects of aging on basal levels of T_4 and cortisol were determined in Experiment 1. For this purpose, each dog was bled once each day at 1100 hr for 4 consecutive days. The effects of aging on the release of T_4 and cortisol during exposure to low ambient temperatures were determined in Experiment 2. Each dog was exposed to four ambient temperatures of 22 (Control), 10, 4, and -5°C in an environmental chamber. A rest period of 4 days was allowed between any two consecutive exposures. Blood samples were collected at 0 hr before and at 0.5, 1, 2, 3.5, and 5 hr during each exposure. At the end of the 5-hr exposure, the temperature was raised to the control level (22°C) and two more blood samples were collected at 1 and 3 hr postexposure. With the control (22°C), no change in temperature was made during the entire 8-hr period.

Blood samples (2.5 ml) in both experiments were collected by cephalic venipuncture into heparinized test tubes and centrifuged immediately in a refrigerated centrifuge for separation of plasma and cells. Plasma fractions were stored frozen for determinations of T_4 and cortisol by RIAs. Total plasma T_4 concentrations were determined with the use of solid-phase RIA reagents (Abbott Laboratories, North Chicago, IL). This assay has cross-reactivities of 0.2 and 1.9% with triiodothyronine (T_3) and reverse T_3, respectively. The standard curve was parallel with the standard curve obtained with various volumes of plasma taken from a pool of canine plasma. This plasma was run as a reference in each assay. Based on T_4 concentrations of this plasma in 46 assays, the inter- and intraassay variabilities of the RIA averaged (±SD) 6.9 ± 2.1 and 4.9 ± 1.0%, respectively. The assay had a sensitivity of 0.36 μg/dl and an average recovery of 96.01 ± 3.1% for various amounts of T_4 added to dog plasma stripped of T_3 and T_4. For cortisol RIA, label was obtained from New England Nuclear (Boston, MA) and antiserum from Radioassay Systems Laboratories Inc. (Carson, CA). The antiserum had cross-reactivities of 11.4, 8.9, and 1.6% with 21-desoxycorticosterone, 11-desoxycortisol, and corticosterone, respectively. It had cross-reactivities of 0.01 to 0.7% with pregnenolone, cholesterol, and 18 other steroids which included various estrogens, progestins, androgens, and glucocorticoids. The standard curve ranged from 7.8 to 1000 pg and was parallel with the standard curve obtained with various volumes of dog plasma. A correlation coefficient of 0.91 was obtained between various amounts of cortisol added to charcoal-treated steroid-free dog plasma and the amounts of cortisol measured in the assay. The inter- and intraassay variabilities averaged (±SD) 4.3 ± 1.9 and 3.1 ± 0.9%, respectively. These estimates of variabilities were based on cortisol concentrations in samples from a pool of dog plasma; this plasma was run as a reference in all assays. Methodological details of this assay are similar to those of other steroid assays (21), except that diluted plasma samples were incubated at 98°C for 15 min to denature the cortisol-binding proteins. Two-way analysis of variance and the Student's *t* test were used to analyze differences between sampling times within groups and between groups.

General. A number of steps were taken to exclude unwanted variables from the experiments. To avoid the effect of breed and strain variability on hormone levels (especially in Experiment 1), all dogs were obtained from a genetically homogeneous colony and had been maintained in our animal quarters under uniform conditions for a number of years. All the dogs were examined carefully to make sure that they were free from any illness. None had a history of any diseases, including those related to the thyroid and adrenal cortex. The dogs had not received in the past and were not receiving during the experiments any drug treatment. The old and adult dogs were accustomed to the bleeding procedure. No physical or chemical restraints were used during blood collection from these dogs. The puppies were held in hands for a brief period (less than 2 min) during blood collection. They became familiar with the bleeding procedure a number of days before the experiment. To prevent novel surroundings and procedures from affecting hormone levels, no changes in feeding or cage-cleaning routines were permitted during the experiments and all animals were made familiar with the environmental chamber for a number of days. To avoid any circadian effects, all dogs were exposed to the various temperatures and bled during exactly the same

time interval (1100 to 1900 hr). To prevent the effects of stages of the estrous cycle, adult dogs were used during anestrus; the old dogs were not cycling and were in anestrus. Hematocrit was checked frequently to make sure it was not affected by blood sampling.

Results. *Effects of aging on basal plasma T_4 and cortisol levels.* Figure 1 shows basal plasma levels of T_4 (top) and cortisol (bottom) in the three age groups. Mean (±SE) T_4 levels in the old dogs (2.8 ± 0.1 μg/dl) were significantly ($P < 0.001$) lower than those in the adults (4.2 ± 0.2 μg/dl) and puppies (4.4 ± 0.2 μg/dl). T_4 levels in the adults were lower

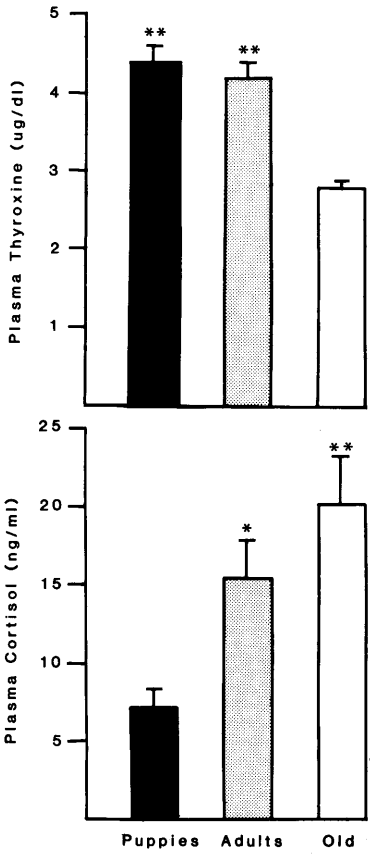

FIG. 1. Changes in plasma thyroxine (T_4) and cortisol concentrations during aging in the dog. Top: Plasma T_4 levels in the old dogs were lower ($P < 0.001$) than those in the adults and puppies. Bottom: Plasma cortisol levels were higher in the adults ($P < 0.005$) and old dogs ($P < 0.001$) than those in the puppies. The difference in cortisol concentrations between the adults and old dogs was not significant. (Bars = means; lines on bars = SE of the mean.)

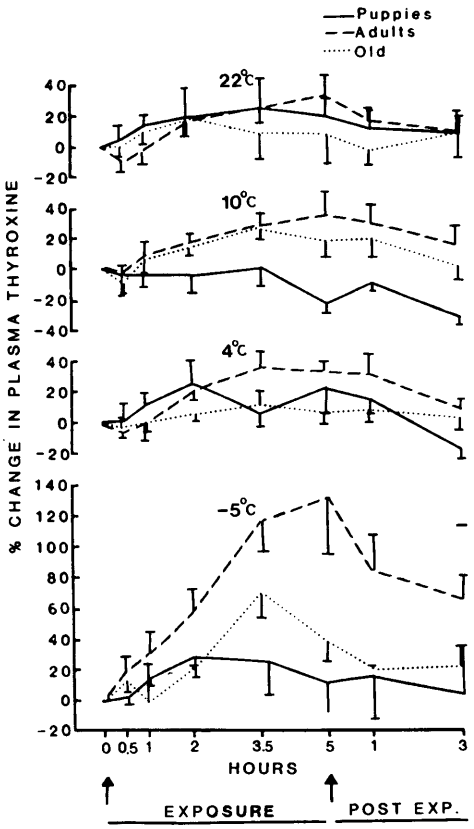

FIG. 2. Effects of aging on mean percentage changes in plasma thyroxine in the dog during exposures to 22 (control), 10, 4, or −5°C. Each exposure lasted 5 hr after which the temperature was raised to 22°C. The 0-hr blood sample was collected just before each exposure.

but not significantly different from those in the puppies. Plasma cortisol concentrations in the old dogs (21.1 ± 3.1 ng/ml) were significantly higher ($P < 0.001$) than those in the puppies (7.2 ± 1.1 ng/ml). Cortisol levels in the adults (15.4 ± 2.4 ng/ml) fell between those in the old dogs and puppies but were significantly different ($P < 0.005$) only from those in the puppies.

Effects of aging on plasma T_4 responses to low ambient temperatures. Percentage changes in plasma T_4 concentrations at room temperature (22°C) and during exposure to three low ambient temperatures (10, 4, and −5°C) are shown in Fig. 2. No significant changes occurred in any of the three age groups at 22°C or during exposure to 10 or 4°C. Exposure to −5°C, however, produced mean (±SE) per-

centage increases of 61.5 ± 9.5, 116.4 ± 13.9, and 130.8 ± 31.3 at 2, 3.5, and 5 hr respectively in the adult dogs. T_4 levels declined gradually when the temperature was elevated to 22°C but were still more than 70% above preexposure levels 3 hr after the end of exposure. In the old animals, exposure to −5°C raised T_4 levels slowly. By 3.5 hr, they reached a maximum of about 70% above the preexposure level and showed a gradual decline during the remaining exposure and postexposure periods. In contrast to the adult and old animals, the puppies showed no significant change in plasma T_4 during exposure to −5°C.

Effects of aging on plasma cortisol responses to low ambient temperatures. Percentage changes in plasma cortisol concentrations in the three age groups at 22°C (control) and during exposure to the low ambient temperatures of 10, 4, and −5°C are shown in Fig. 3. No significant changes in plasma cortisol were observed in any of the three groups during exposure to 22, 10, or 4°C. Exposure to −5°C had no effect in the puppies and old dogs but produced a rapid increase in cortisol concentrations in the adult dogs. This increase was more than 280% above preexposure levels by 1 hr and remained significant ($P < 0.05$) during the remaining 4 hr of exposure. Plasma cortisol levels decreased sharply when the exposure to −5°C was terminated and ambient temperature was raised to the control level (22°C).

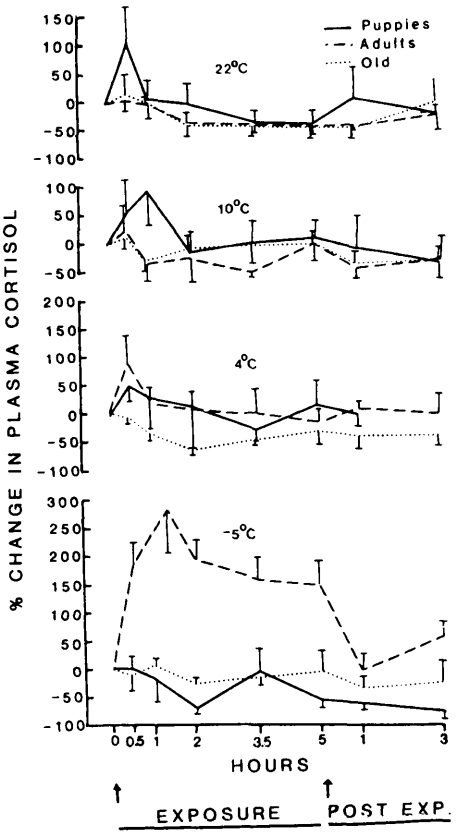

FIG. 3. Effects of aging on mean percentage changes in plasma cortisol in the dog during exposures to 22 (control) 10, 4, or −5°C. Each exposure lasted 5 hr after which the temperature was raised to 22°C. The 0-hr blood sample was collected just before each exposure.

Discussion. These results demonstrate that, with advancing age, plasma concentrations of T_4 and cortisol in the dog change in opposite directions, with a decrease in T_4 and an increase in cortisol. It is possible that these changes have a cause and effect relationship as indicated by the evidence that high levels of glucocorticoids inhibit basal levels of thyroid hormones in dogs (1), man (2), and rats (3). The exact mechanism of this inhibition is not clear, although glucocorticoid treatment has been shown to produce histological changes in the thyroid (1), inhibit the release of TSH and TRH, and suppress TSH responses to TRH in man and rats (4–6).

The fact that plasma T_4 declined only insignificantly in adult dogs as compared to puppies, but decreased by more than 30% between adulthood and old age, indicates that the age-related decline in plasma T_4 is a gradual process, the cumulative effect of which becomes significant only in the later part of life. In a recent study in man, plasma T_4 showed no change up to puberty but decreased significantly during middle and old ages (13). Our results also agree with other studies of man and other species (7–12). As described elsewhere in this paper, nutritional, disease-related, genetic, and environmental factors that can affect T_4 levels were excluded from these experiments and the results have been confirmed in another study of dogs (Gonzalez and Quadri, unpublished). Furthermore, in view of the reported age-related differences in endocrine responses to stress (8, 22–24), every attempt was made in the present study to ex-

clude stress during blood collection. No comparable study of the dog is available but our results are also supported by the fact that hypothyroidism is one of the most common ailments of old age in the dog. Although none of the old dogs used in this study were clinically hypothyroid, the fact that they had lower T_4 levels than the adults raises the possibility of their becoming hypothyroid with further passage of time. Low thyroid activity in old age is also supported by other lines of evidence, which indicate that metabolic activities which are influenced by thyroid hormones begin to decline with advancing age in man and other species (25, 26).

The absence of a T_4 response to cold in the 8-week-old puppies and the greatly subdued and short-lived response in the old animals indicate that the capacity to respond to low ambient temperatures by an increase in T_4 has not yet developed in the very young and is on the decline in the aging animals. There is disagreement on T_4 and TSH responses to cold in man (27, 28) and no studies have been done on the effects of aging on these responses. In the rat, cold increases these hormones but to our knowledge only one study has examined how these increases are affected by aging (8). In this study, in concurrence with our results, cold induced a marked increase in plasma T_4 in 6- to 8-month-old rats but failed to do so in 20- to 24-month-old rats.

The reasons for the low T_4 levels and attenuated T_4 responses to cold in old animals are not clear. To what extent these results were influenced by age-related changes, if any, in the binding proteins or TSH secretion, or by thyroid autoantibodies is not known. Also, there is little consensus on the nature and locus of age-associated changes in the hypothalamo–pituitary–thyroid axis. There is evidence that aging is accompanied by atrophy, fibrosis, and other degenerative changes in the thyroid (29–31) and by increases in the intrathyroidal and intrahepatic conversions of T_4 to T_3 (10). In contrast, so far there is little evidence that an age-related decrease in T_4 is due to impairments at the pituitary or hypothalamic levels (8). Thus, the effects of aging on the synthesis and release of TRH are not known and conflicting reports have appeared on age-related changes in TSH responses to TRH in the rat (7) and man (32, 33). A recent study in the rat indicates no effect of age on TRH-induced TSH release except with very low doses of TRH which elicit greater responses in older animals (11). Similarly, TSH responses to cold in old rats are similar to those in young ones (8).

Our observation that with increasing age there is a gradual increase in basal cortisol levels in the dog is similar to the recently reported progressive increase in corticosterone in the aging rat (17, 18). However, no age-related increases in glucocorticoids were found in a number of other studies (15, 16, 19). All the reasons for these contradictory results are not clear, but some of them may be related to the use of an inadequate number of age groups (17), the stresses of ether treatment and animal handling (19), a general lack of control of factors that affect cortisol levels (18), and other methodological difficulties. It can be argued that the increase in cortisol levels in the old animals that we observed by sampling at one specific time on separate days was due to a shift in cortisol rhythm rather than to a change in the secretion rate. We have, however, discounted this possibility since in a recently completed study we were unable to detect a shift in cortisol rhythm in old dogs (Palazzolo and Quadri, unpublished). Here it is noteworthy that some of the symptoms of chronic hyperadrenocorticism, such as increases in blood cholesterol and lipids, a tendency to develop diabetes, decreases in immunological capacity, and other impairments in metabolic and neuronal functions, are also frequently seen in senescence (17). However, more data are needed to decide whether this relationship between some of the symptoms of senescence and hyperadrenocorticism is causal.

The cold-induced increase in plasma cortisol in adult dogs is consistent with a number of other reports in various species (34–36). Glucocorticoids and thyroid hormones restore thermogenic responses and cold tolerance in hypophysectomized and adrenalectomized animals and are important for survival in the cold. Our results indicate that very young and old animals are unable to increase plasma glucocorticoids in response to cold stress. It is not known whether this is due to impairments at the hypothalamic, pituitary, or adrenal level

or to differences in the magnitude of cold stress perceived by these age groups.

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