Circannual changes in free thyroxine, prolactin, testes, and relative food intake in woodchucks, *Marmota monax*

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Concannon, Patrick W., V. Daniel Castracane, Richard E. Rawson, and Bud C. Tennant. Circannual changes in free thyroxine, prolactin, testes, and relative food intake in woodchucks, *Marmota monax*. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46): R1401–R1409, 1999.—Woodchucks (n = 12–14/group) with circannual cycles entrained to northern versus southern hemisphere photoperiods were assessed monthly for 16 mo. Changes in serum total triiodothyronine (TT_3), free thyroxine (T_4), total thyroxine (TT_4), and prolactin were determined in a subset of five animals per group. Metabolic hormone results were examined in relation to changes in body weight, food intake, and serum testosterone (n = 12–14/group). Seasonal changes in each parameter were similar in both groups as were nadir and peak TT_3 (162 ± 6 and 392 ± 12 ng/ml, respectively), free T_4 (19 ± 2 and 86 ± 7 ng/ml, repectively), TT_4 (3.2 ± 0.2 and 8.0 ± 0.5 ng/ml, respectively), and prolactin (0.6 ± 0.1 and 14 ± 2 ng/ml, respectively). In late winter and early spring, simultaneous increases in both free T_4 and prolactin were associated with *1*) a large increase in food intake, *2*) a decline in body weight to nadir values, *3*) a corresponding negative energy balance, *4*) a peak and decline in serum testosterone, and *5*) a modest increase in TT_4 and large decline in serum TT_3. Low levels of free T_4 and prolactin were observed in summer when energy balance was very positive. The results demonstrate that, in woodchucks, serum T_4 and prolactin undergo seasonal changes during annual cycles entrained by photoperiod. The results suggest that changes in free T_4, acting as a calorigenic hormone, and changes in both T_4 and prolactin, potentially acting as lipolytic, antilipogenic, and/or orectic hormones, are likely involved in the mechanisms underlying the corresponding seasonal changes in food intake, fat metabolism, and energy balance in this species. Their potential roles in gonadal regression and recrudescence are less clear.

food intake; circannual cycles; testis; testosterone; thyroid; adipose

THE ENDOGENOUS CIRCANNUAL cycle of woodchucks involves large changes in gonadal function, food intake, and body weight. The cycle free runs at 8- to 12-mo intervals (7, 10) and is entrained and synchronized to 12 mo by natural changes in photoperiod (5). The woodchuck and other marmotine sciurid rodents undergo several months of deep hibernation under natural conditions. They also appear to require natural changes in photoperiod to entrain the endogenous cycle. In these species, block changes in photoperiod such as persistent long days, persistent short days, and transition from long days to short days have failed to perturb the circannual cycle (10, 22). However, photoperiods involving daily changes in photophase duration that simulate the natural environment have prevented free running in woodchucks (5). Furthermore, woodchucks maintained under conditions simulating a southern hemisphere (austral) photoperiod show an endogenous cycle that is advanced by 6 mo relative to woodchucks maintained in a northern hemisphere (boreal) photoperiod (5).

The circannual cycle of the woodchuck has been described in terms of seasonal changes in testis size, serum testosterone, ovarian activity and pregnancy, food intake, body weight, and body fat content (1, 5, 6, 10, 31). However, the extent and time course of seasonal changes in prolactin, total thyroid hormone, or free thyroxine (T_4) have not been examined in detail. In radioimmunoassay studies on wild ground squirrels, an increase in plasma prolactin occurred during the spring (13). Prolactin can have both pro- and antifertility effects in male rodents (3, 30). In some species, prolactin can also stimulate both food intake and lipolysis (2, 12). A seasonal elevation in prolactin in the woodchuck would be of interest as a possible effector of seasonal changes in gonadal and metabolic activity.

Histologically, the woodchuck thyroid appears inactive in winter, most secretory in appearance in the spring, apparently nonsecretory in the summer, and as having some synthetic activity in the fall (31). In woodchucks examined at four times of the year, total thyroxine (TT_4) in serum was reported to be highest in winter (when the animals are least active) and lowest in summer, whereas free T_4 was lowest in the winter and highest in the spring (31). This finding is largely due to the presence of much higher levels of thyroxine-binding globulin (TBG) in the winter than in the summer (31). Because changes in thyroid hormone status are likely to explain, in part, seasonal changes in food intake and energy efficiency, information on the time course of changes in free T_4 relative to changes in food intake and body weight would be of interest.

In the present study, therefore, serum samples obtained monthly for 16 mo from male woodchucks entrained to boreal and austral photoperiods were assayed for prolactin, total triiodothyronine (TT_3), and free T_4 as well as TT_4, and the results were considered in relation to previously observed changes in body weight, estimated daily food intake, and testis function (5). The austral-versus-boreal-photoperiod paradigm

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was used to ensure that changes that appeared to be circannual were in fact photoperiod-entrained circannual changes occurring independent of chronological age or possible environmental or husbandry changes unrelated to the biology of the annual cycle. Males were used to avoid any pregnancy-specific changes in prolactin or T_4 binding.

MATERIALS AND METHODS

Animals. Male woodchucks born in March in the wild and captured at 2.5 or 14.5 mo of age were initiated on austral ($n = 14$) or boreal ($n = 14$) photoperiods in June. After 2 yr, the austral photoperiod males and cohort females had circannual changes in gonadal activity and body weight that were advanced by ~6 mo relative to those of boreal controls, as previously reported (5). Two males died due to cardiac infarction and aortic aneurysm. From *month 20* through *month 35* of photoperiod treatment, the 12–14 remaining pair-housed males in each group were used for the present study. During this 16-mo period, testis size, serum testosterone, body weight, estimated daily food intake (g/day), and relative food intake ($g \cdot kg^{-1} \cdot day^{-1}$) continued to be monitored monthly in all animals using methods previously described in detail (5). However, body weights were not recorded for the third month of study. Serum samples obtained monthly from five males in each group were assayed to assess changes in prolactin and thyroid hormone status. Serum samples were obtained under ketamine (50 mg/kg) and xylaxine (5 mg/kg) anesthesia by femoral venipuncture as previously reported (5).

Throughout the present study, photoperiods continued to simulate those of 42°N and 42°S with daily increases or decreases in day length of 0–4 min/day. Solstices occurred on December 21 and June 21 with 9 and 15 h of light per day, respectively. Equinoxes occurred March 18–24 and September 18–25. Room temperatures were maintained between 20 and 23°C, and food (Woodchuck pellets, Agway, Syracuse, NY) and water were available ad libitum. Because animals were typically pair-housed in cages, food intake was determined on a per-cage basis and was then divided by the number of animals to obtain a value for grams per animal per day. Food intake was measured on each of five sequential days each month, and the average of those values was designated the average daily food intake (g/day) for the month. Relative food intake ($g \cdot kg$ body $wt^{-1} \cdot day^{-1}$) was determined monthly as average daily food intake per cage relative to total body weight per cage. Energy balance can vary significantly over short periods (4). Therefore, body weight and food intake data were obtained at monthly intervals, a period that is reasonably long enough to make meaningful assessments of energy balance. An increase in body weight over a 1-mo period was considered evidence of positive energy balance, whereas a decrease in body weight over the same period was judged to indicate negative energy balance.

Hormone assays. Serum samples were maintained frozen until assayed. Serum samples from each male were assayed for testosterone content in duplicate using the RIA method previously described and validated for woodchucks (7).

Serum samples obtained monthly from five males in each photoperiod were assayed for concentrations of TT_3, free T_4, and prolactin. All 10 males were housed in different cages. Thyroid hormone concentrations were measured in duplicate in woodchuck serum using commercial assay reagents. The assays for TT_3 (DPC TT_3 RIA) and free T_4 (DPC Free T_4 RIA) were performed as directed by the manufacturer [Diagnostic Products (DPC), Los Angeles, CA]. In both rats and woodchucks, thyroid hormone is bound to TBG, and the free T_4 assay used in this study has been used previously to assay free T_4 in woodchucks as well as rats (23). TT_3 was used as a means of monitoring TBG binding of thyroid hormone, because distribution studies using radio-labeled hormones in woodchucks determined that ~80% of triiodothyronine (T_3) is bound to TBG, whereas up to 60% of T_4 can be bound to albumin and prealbumin (31). After completion of free T_4 and TT_3 assays, TT_4 was assayed in the same samples to determine to what extent the human TT_4 assay results might reflect the reported seasonal changes in TBG that appeared evident in the TT_3 results or reflect presumed seasonal changes in thyroid hormone secretion. The assays for TT_4 (DPC TT_4 RIA) were also performed as directed by the manufacturer (DPC).

In all three thyroid hormone assays, serial dilutions of two or three woodchuck serum samples were parallel and did not differ from those of the standard curve ($P = 0.65–0.92$). In addition, serum samples collected before versus 6 h after thyroid-stimulating hormone (TSH; Sigma, 2 μg/kg im) in five woodchucks were assayed for TT_3 and free T_4 content. In response to TSH, there were consistent increases ($P < 0.05$) in free T_4 (105 ± 16 vs. 70 ± 8 ng/dl) but not in TT_3 (214 ± 15 vs. 181 ± 26 ng/dl). Levels of TT_3 and free T_4 did not change after injection of gonadatropin-releasing hormone (GnRH; 1.0 μg/kg im) or ACTH (5 IU/kg im). For both free T_4 and TT_3, all 16 monthly samples from each animal were included in the same assay, and for TT_4, the 13–16 available serum aliquots for each animal were included in the same assay. Samples from one or more animals in each photoperiod group were included within each assay. On the basis of serum samples included in each assay, the within- and between-assay coefficients of variation were 7 and 12%, respectively, for TT_3, 10 and 19%, repectively, for free T_4, and 11 and 16%, respectively, for TT_4.

Prolactin content of serum was determined in triplicate using a canine prolactin (cPRL) assay (A. Parlow, Harbor Hospital, Torrance, CA) performed as previously described for canine plasma samples (14). The assay used a guinea pig anticanine prolactin serum (AFP-1062091) at a dilution of 1:250,000 (500 μl) and a cPRL standard (AFP-2451B-cPRL) for both standard curves (200 μl) and for tracer [I^{125}]cPRL (100 μl) prepared using mild chloramine-T iodination (8 μg/2 μg cPRL, 1.0 min). The assay was evaluated for use in woodchucks based on parallelism, recovery of hormone, and biological responses. Displacement curves for serial dilutions of each of two woodchuck serum samples (10.2 and 3.1 ng/ml) were not different ($P > 0.84$) from those for the canine prolactin standards. Serum samples from groups of five woodchucks before and 30 min after thyrotropin-releasing hormone (TRH; 2 μg/kg im) or GnRH (1 μg/kg im) yielded prolactin concentrations that demonstrated prolactin release by the prolactin secretagogue TRH (5.4 ± 0.9 vs. 2.9 ± 0.5 ng/ml), but not by GnRH (1.2 ± 0.4 vs. 1.0 ± 0.3 ng/ml). Prolactin levels determined for samples obtained in April from five lactating woodchucks (14.5 ± 1.6 ng/ml) were higher ($P < 0.05$) than those from nonlactating animals (6.9 ± 2.3 ng/ml) and higher ($P < 0.05$) than those determined for the same animals in October (0.6 ± 0.2 ng/ml). In the present study, all samples from each animal were included in the same assay. Samples from one or two animals in each photoperiod group were included in each assay. On the basis of two serum samples (2.2 and 20.1 ng/ml), the within-assay and between-assay coefficients of variation were 9 and 13%, respectively.

Statistics. Values are reported as means ± SE. Comparisons between groups were made using Student's *t*-test. Differ-

ences between means were considered significant at the $P = 0.05$ level.

RESULTS

Thyroid hormones. Free T_4 concentrations in both photoperiod groups were at intermediate values in the fall and early winter, increased during the late winter and spring, and then declined toward nadir values during the summer (Figs. 1 and 2). Peak free T_4 concentrations ranged from 0.63 to 1.19 ng/dl and averaged 0.86 ± 0.07 ng/dl. Peak free T_4 occurred in the spring in both groups, at April 8 ± 4 days in boreal males and October 14 ± 12 days in austral males. Nadir free T_4 concentrations ranged from 0.08 to 0.26 ng/dl and averaged 0.19 ± 0.02 ng/dl. Nadir free T_4 occurred in late summer in both groups, at August 12 ± 10 days in boreal males and March 15 ± 12 days in austral males.

Peak TT_3 concentrations ranged from 325 to 456 ng/dl and averaged 392 ± 12 ng/dl. Peak TT_3 values occurred during the winter in both boreal (January 10 ± 15 days) and austral (August 10 ± 5 days) woodchucks. Mean concentrations of TT_3 declined continuously during the late winter and spring in both groups and were low in summer before again increasing (Figs. 1 and 2). Nadir TT_3 concentrations ranged from 139 to 184 ng/dl, averaged 162 ± 6 ng/dl, and occurred in summer in both groups, on June 22 ± 10 days in boreal animals and January 21 ± 5 days in austral animals.

Serum concentrations of TT_4 followed a pattern different from that of TT_3 or free T_4 in each photoperiod group (Figs. 1 and 2). TT_4 concentrations in individual animals of both groups were lowest (3.2 ± 0.2) in late summer and autumn, intermediate in winter, and highest (8.0 ± 0.5) in early spring. The values for the ratios of mean peak to mean nadir for thyroid hormones were 2.5 for TT_4, 2.4 for TT_3, and 4.5 for free T_4.

Prolactin. Mean prolactin concentrations were, in general, low in the summer, autumn, and early winter, and elevated for ~3 mo in late winter and early spring (Figs. 1 and 2). Nadir prolactin concentrations ranged from 0.2 to 1.3 ng/ml and averaged 0.6 ± 0.1 ng/ml. On average, nadir prolactin occurred in the autumn in both groups, at September 8 ± 54 days in boreal males and April 9 ± 29 days in austral males. Peak prolactin

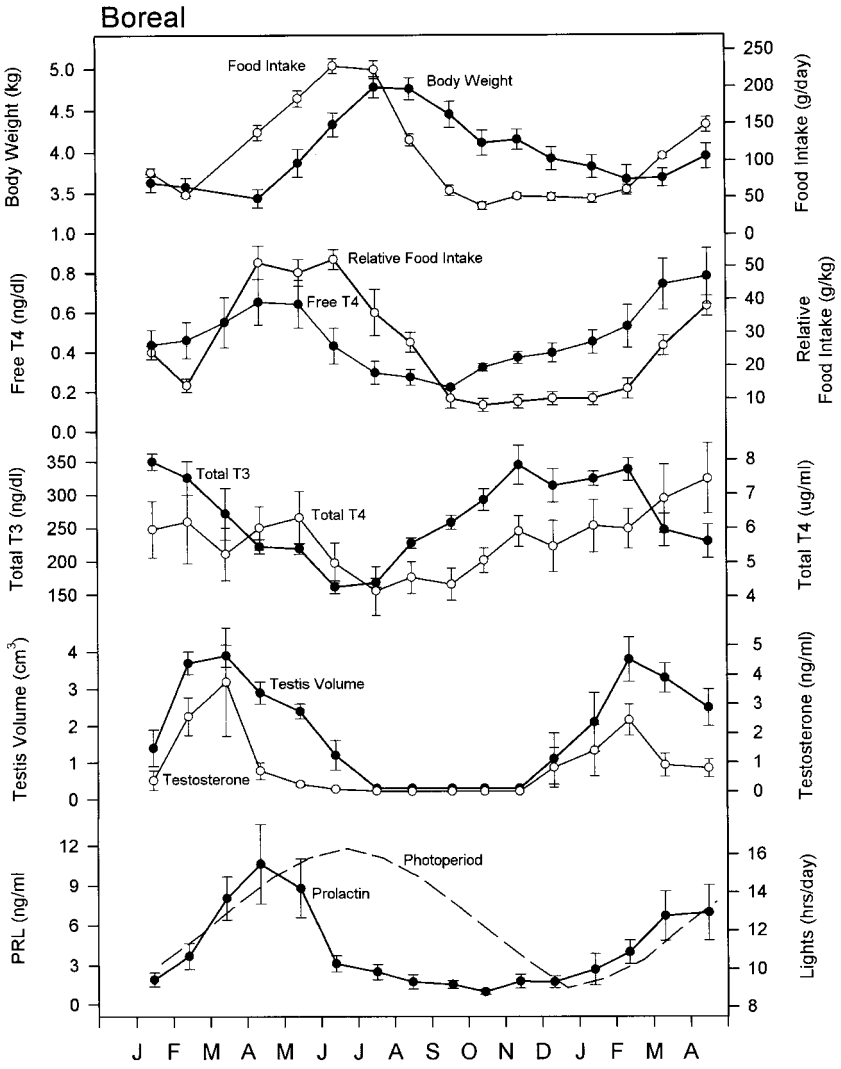

Fig. 1. Boreal data. Mean (±SE) body weights, daily food intake, serum-free thyroxine (free T_4), serum total triiodothyronine (TT_3), serum testosterone, testis size, and serum prolactin in (PRL) groups of male woodchucks during *months 20–35* of exposure to northern hemisphere (boreal)-simulated natural photoperiods shown in relation to seasonal changes in photoperiod provided by daily changes in photophase. Number of observations/mean is 13 or 14 for body weight and testis size, 6–8 for food intake, and 5 for hormone data.

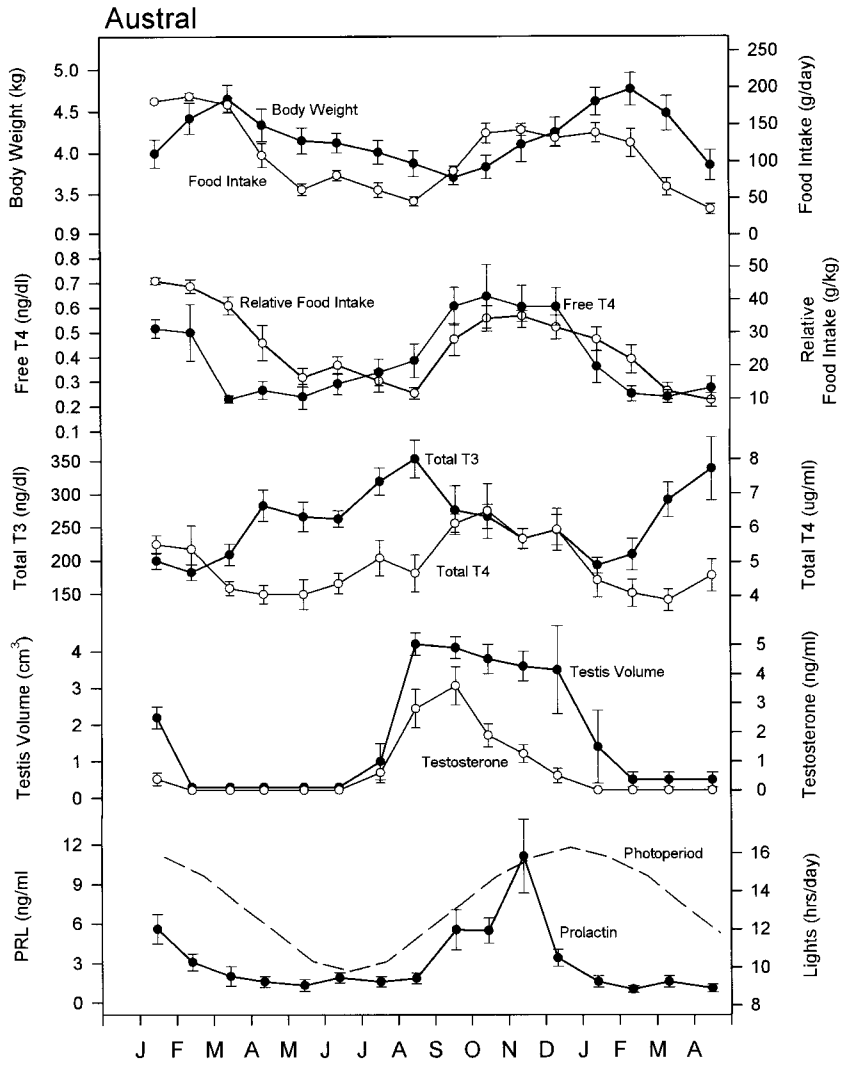

Fig. 2. Austral data. Mean (±SE) body weights, daily food intake, serum-free T_4, serum TT_3, serum testosterone, testis size, and serum PRL in groups of male woodchucks during *months 20–35* of exposure to southern hemisphere (austral)-simulated natural photoperiods and shown in relation to seasonal changes in photoperiod provided by daily changes in photophase. Number of observations/mean is 11 or 12 for weight and testis size, 6 or 7 for food intake, and 5 for hormone data.

ranged from 7.0 to 20.7 ng/ml and averaged 13.7 ± 2.0 ng/ml. Peak prolactin occurred in the early spring in both groups, on April 9 ± 13 days in boreal males and October 15 ± 12 days in austral males, and then declined during the late spring and summer.

Body weights, food intake, and energy balance. Mean body weights were maximal in the summer, in July and August in boreal males and in February in austral males, when free T_4 had declined to near-minimum values (Figs. 1 and 2). Mean body weight was lowest in late winter, in March in boreal males and September in austral males, when free T_4 was increased to maximum or near maximum values. Peak body weights ranged from 3.8 to 6.1 kg, averaged 5.0 ± 0.1 kg (n = 26), and did not differ between austral (5.0 ± 0.2 kg, n = 12) and boreal (4.9 ± 0.1 kg, n = 14) male woodchucks. In both groups, peak body weight occurred in the summer, with mean dates of July 27 ± 5 days in boreal animals and February 2 ± 9 days in austral animals. The dates on which peak body weights were achieved, using the summer solstice as a reference point, did not differ between boreal and austral woodchucks, indicating seasonal coincidence of their respective growth curves. Nadir body weights ranged from 2.7 to 4.7 kg, averaged 3.3 ± 0.3 kg (n = 26), and did not differ between austral (3.5 ± 0.1 kg) and boreal (3.2 ± 0.1 kg) animals. Differences between sequential peak and nadir body weights ranged from 0.7 to 2.4 kg, averaged 1.4 ± 0.4 kg, and represented body weight losses of 12.1–41.9% (25.9 ± 1.3%) and body weight gains of 38–75% (48 ± 3%). Body weights for the five males evaluated for endocrine profiles in each photoperiod group did not differ from those of the larger group of 12–14 woodchucks of which they were a part.

Peak daily food intake averaged 214 ± 9 g/day and occurred in late spring or early summer in both boreal (June 17 ± 5 days) and austral (December 27 ± 10 days) woodchucks. Nadir daily food intake averaged 29 ± 2 g/day (n = 26) and occurred in the winter both in boreal males (December 27 ± 12 days) and in austral males (June 25 ± 15 days). As with body weight, dates at which peak food intakes occurred for the two groups did not differ in relation to the date of the summer solstice. The largest increase in mean food intake routinely occurred when mean free T_4 and prolactin concentrations were at or near maximum. However, mean food intake remained high for 1–2 mo after

obvious declines in both free T_4 and prolactin (Figs. 1 and 2).

Peak relative food intake averaged 47 ± 3 g·kg^{-1}·day^{-1} and occurred in late spring, at May 10 ± 8 days in boreal males and December 2 ± 12 days in austral males, ~6 wk before peaks in body weight (Figs. 1 and 2). The most pronounced periods of positive energy balance, involving large increases in body weight during a decline in relative food intake, occurred in early summer in each group (Figs. 1 and 2.). This period coincided with a large decline in mean free T_4 and prolactin concentrations in both groups. Nadirs for relative food intake averaged 5 ± 0.5 g·kg^{-1}·day^{-1} and occurred in late autumn or early winter, at November 9 ± 14 days in boreal males and July 6 ± 17 days in austral males. The nadir for mean relative food intake preceded the nadir and initial increase in mean body weight by 1–3 mo in both groups (Figs. 1 and 2). This period coincided with mean free T_4 and prolactin concentrations increasing to near maximum values in both groups.

Testes size and serum testosterone. There were no differences between the subsets of five males and the larger groups of males in mean peak values for testis volume (data not shown) or serum testosterone [4.1 ± 0.4 (n = 10) and 4.8 ± 0.8 ng/ml (n = 26), respectively] or in the times of their occurrence. The testosterone results for all animals are shown (Figs. 1 and 2). Initial increases in testosterone ≥0.3 ng/ml occurred in late winter in both groups, on January 22 ± 5 days (n = 12) in boreal males and August 13 ± 7 days (n = 14) in austral males. In both groups, testosterone decreased most rapidly in late winter and early spring when concentrations of free T_4 and prolactin were at maximum or near-maximum values (Figs. 1 and 2).

DISCUSSION

The present results detail the timing of seasonal changes in prolactin and thyroid hormone status during photoperiod-entrained annual cycles in woodchucks and establish their relationship to changes in energy balance, body weight, food intake, and serum testosterone. The patterns of free T_4, TT_3, and TT_4 were to a large extent those predicted by earlier more limited studies (31). The transient late winter and early spring increase in prolactin concomitant with that of free T_4 is a new observation. The relative magnitudes of weight gain were similar to those previously reported for woodchucks and other sciurid rodents (10) and presumably consist almost entirely of fat (9). Consequently, the observed periods of pronounced negative and positive energy balance in both groups likely involved a transition from a primarily lipolytic state to a primarily lipogenic state as well as a change in resting metabolism.

Precise analysis of energy balance is achieved through measurement of energy input (i.e., the caloric value of metabolized food) and output (usually determined by measurement of heat production) (4). Although neither of these variables was measured in the present study, energy balance, the difference between energy input and output, can be assessed by measurement of body weight changes, because any imbalance between energy input and output will result unavoidably in altered body weight. Furthermore, some insight into potential mechanisms underlying observed changes in energy balance can be deduced from food intake data.

Body weight and relative food intake of most nonhibernating mammals, as measured over reasonably long periods, is remarkably constant (4). In contrast, body weight of woodchucks in late winter declined, whereas food intake and relative food intake increased, i.e., woodchucks were in a period of negative energy balance despite increased energy input. Energy expenditure, then, must have been increasing. In the natural environment during spring, cold ambient temperatures and increased locomotor activity associated with foraging would contribute to increased energy expenditure. In the laboratory setting, however, room temperature is constant, locomotor activity is limited, and foraging is unnecessary. On the other hand, studies have shown that changes in metabolism occur at a level that is more fundamental than behavior or environmental adaptation. Resting CO_2 production is known to have a circannual cycle (1), and resting O_2 consumption of laboratory woodchucks was higher in the spring than in the fall (7.3 ± 0.3 vs. 4.3 ± 0.2 ml O_2·min^{-1}·kg^{-1}) under conditions similar to those of the present study (23). Therefore, a major component of the increase in energy expenditure in spring can be attributed to circannual changes in basal metabolism. This conclusion is consistent with the observed increase of free T_4 during early spring.

Food intake is correlated with body weight, but measures of relative food intake take this relationship into account. Relative food intake of woodchucks increased during late winter and early spring, indicating that food consumption was greater than could be accounted for on the basis of body weight alone. Relative food intake remained at a peak value during spring and early summer, and body weight continued to increase. This result suggests that either food intake was greater than that required for maintenance of a stable body weight or metabolic rate had declined to such an extent that a larger proportion of energy intake could be stored as fat. To determine changes in the relative contributions of these two processes would require concurrent measurements of metabolic rate, food intake, and unabsorbed food as well as body weight.

In late summer, body weight remained constant, whereas food intake decreased. Energy expenditure, then, must have been declining. This is consistent with the observed decrease in free T_4 during this period and with the findings of Bailey (1), who reported that metabolic rate of resting woodchucks declines between May and February.

Thyroid hormone is calorigenic by virtue of its effects on mitochondrial function in most tissues, especially adipose tissue (21), and is lipolytic due to effects both on hormone-sensitive lipase activity directly and on adipose cell responses to catecholamines (16). The magni-

tude of observed changes in free T_4 and the timing of those changes were different from those of TT_3 and TT_4. The concentrations and patterns of TT_3 and TT_4 in the present study, in which the animals were not induced to undergo deep hibernation, were generally similar to those reported for a radioimmunoassay study of woodchucks in the 7-mo period following emergence from an induced hibernation (31), with few exceptions. For each hormone, the lowest levels measured were essentially the same in both studies and were observed in summer. However, the transient early-spring increase in TT_4 was not observed in the previous study, and in the present study peak concentrations of both hormones were somewhat higher. These differences could be due to differences in the radioimmunoassay used, the limitation of only monthly samples in both studies, or the degree of synchrony among animals.

Free T_4 has not previously been reported in serially collected samples in woodchucks or related species. The pattern of free T_4 observed in both photoperiod groups in the present study agrees with our observation of higher concentrations of free T_4 in spring than in autumn in other groups of boreal and austral photoperiod woodchucks (23). In that study, higher free T_4 was associated with correspondingly higher levels of oxygen consumption at the single time points studied. The present results further demonstrate a rapid decline in free T_4 in late spring and early summer, nadir concentrations in early autumn, and a slow but evident increase in early winter. The occurrence of a greater than fourfold change in free T_4 during the annual cycle suggests a change in thyroid hormone status equivalent to a transition from a hyperthyroid state to a hypothyroid state in those species in which free T_4 concentrations are more closely regulated.

Our information on changes in thyroid hormone status remains incomplete in that available sample size did not permit measurement of free T_3 in addition to TT_3, free T_4, and TT_4. Nevertheless, the observed changes in free T_4 can be considered to reflect the major changes in functional thyroid hormone status. In humans and other species studied, free T_4 is the major determinant in the peripheral circulation of total active thyroid hormone, because it is present in far greater amounts than free T_3 and because most of intracellular T_3 is derived from T_4 uptake from the circulation (17). Similarly, the observed changes in TT_3 are likely to reflect changes in TBG binding of thyroid hormones to a greater degree than changes in TT_4. In woodchucks, as in humans, the proportion of T_3 bound to TBG is greater than that of T_4, and a greater proportion of T_4 is bound to albumins (17, 31). Consequently, the period in early spring in which TT_3 declined while free T_4 increased in both groups presumably reflects a decrease in circulating TBG at that time. That is in agreement with the observation that serum TBG in woodchucks is highest in winter and lowest in summer (31). This decline in TBG during the late winter, spring, and early summer could involve decreased hepatic synthesis, increased clearance, or both. Similarly, the period in which TT_3 appeared to increase at a greater rate than free T_4 in the late summer and autumn presumably reflects an increase in circulating TBG due to increased synthesis, decreased clearance, or both. However, it is important to recognize that some of the changes in TT_3 might reflect changes in peripheral deiodinase activity, which was not evaluated in this study.

Factors regulating liver TBG production are poorly understood, and TBG has only recently been known to be produced in some rodents, including rats, mice, and woodchucks (29, 31). Evidence to date suggests that liver TBG synthesis involves multiple isoforms and is reduced by thyroid hormone, corticosteroids, androgens, and the cytokine interleukin-6 as well as an unidentified pituitary factor (11). Whether or not the concurrent increases in testosterone, free T_4, or possibly even prolactin might play roles in the decline in TBG (and resulting decline in TT_3) in woodchucks merits investigation. Prolactin has mitogenic and growth-stimulating effects on liver cells, but possible effects on TBG production have not been studied.

There are several possible reasons why the observed changes in TT_4 were different from those of TT_3 in addition to differences in factors regulating production, utilization, and clearance. In addition to high-affinity globulins, thyroid-binding proteins in woodchucks include albumin and prealbumin (31) if not lipoprotein as in some species (17). In woodchucks, a large portion of T_4 is bound to albumin in the spring and summer, whereas in the fall and winter, most of the binding is to TBG (31). Therefore, changes in TT_4 reflect, in addition to changes in secretion, not only changes in TBG but also potential changes in the other T_4-binding proteins. TT_4 concentration also reflects changes in thyroid hormone secretion to a greater extent than does that of TT_3, because all circulating T_4 comes from the thyroid gland and most circulating T_3 comes from peripheral deiodination. It is also possible that the human total thyroid hormone assays used in this study differ in the extent to which antibody affinity is higher than the affinity of various serum-binding proteins for the particular ligand. Therefore, in the case of T_4, more than T_3, this might result in a variation in the proportion of total hormone measured as TBG levels change, because in other species TBG has a greater affinity for T_4 than for T_3 (17). Nevertheless, for the changes in TT_4 and TT_3 observed in this study, the following can be considered. Increasing or unchanged concentrations of TT_4, while those of TT_3 were declining in the spring, probably reflect increased synthesis during a decline in TBG but might also reflect increases in other binding proteins. Declines in TT_4, while TT_3 was also declining in summer, were probably due to a decrease in TBG in addition to any decrease in synthesis. Finally, declining or unchanging concentrations of TT_4, while those of TT_3 were increasing in early autumn, probably represent decreased T_4 synthesis.

If these assumptions are correct, then thyroid hormone results for both photoperiod groups suggest three phases of thyroid hormone status that merit further study. First, there is an apparent increase in thyroid hormone secretion for ~2–3 mo in late winter and early

spring, during a concomitant decrease in TBG concentrations, resulting in an elevation in free T_4. This period is reflected in increased TT_4 and free T_4 and declining TT_3. Second, there is apparently a large decrease in thyroid hormone secretion in the summer and autumn, with an associated autumnal increase in TBG production. This is reflected in free T_4 and TT_4 concentrations reaching nadir values while TT_3 is increasing. Third and finally, there is apparently a period of slow increases in TT_4 and, to some extent, free T_4 in late autumn and early winter, whereas TT_3 remains high. Presumably, these changes are associated with sustained increases in synthesis of T_4 and of TBG.

Information on the control of associated changes in TSH secretion and hepatocellular activity would be helpful in explaining what appears to be a transient hyperthyroid state in early spring. Such changes in thyroid hormone secretion and concentrations are in agreement with the observation that the histological correlates of thyroid gland activity in woodchucks are increased in the spring and decreased in the summer (15) and with available information on changes in serum concentrations in TBG (31). The late winter and early spring increase in free T_4 is undoubtedly the cause of the higher resting metabolism measured in woodchucks in early spring compared with early autumn (23). However, concomitant measurements of resting metabolism and free T_4 would be needed to confirm the extent to which metabolism mirrors the low free T_4 levels observed in the summer. It would appear likely that the increased thyroid hormone in late winter and early spring to some extent also stimulates the concurrent increases in food intake, at least indirectly as a result of calorigenesis and an increased energy requirement, as suggested for its efficacy in increasing food intake in reindeer (26) and in rats (21).

It is unclear whether the observed autumnal and early winter increases in TT_4 and free T_4 actually reflect small increases in thyroid hormone synthesis or instead reflect a decline in a binding protein such as albumin that alters the TT_4 assay results and increases apparent free T_4 concentrations. Whether or not the apparent early winter increases in free T_4 occur, or occur at a different time, under natural conditions of true hibernation would also be of interest. The results also raise the question of a potential role for such spontaneous increases in free T_4 in the mechanism of emergence from hibernation and from the hibernation-like conditions experienced by laboratory woodchucks in the autumn and winter. Unfortunately, temperature was not monitored in these groups of animals, but, in similar groups of austral and boreal photoperiod-exposed woodchucks, we have observed that rectal temperature fluctuates seasonally and tends to be lowest in early autumn and to increase slightly during late autumn and winter (Concannon and Rawson, unpublished observations). Those observations would suggest that the slow increases in free T_4 measured during the winter in the present study are biologically significant.

The decline in serum testosterone in early spring occurred while free T_4 and prolactin were increasing to peak concentrations. The relationships may be more than coincidental, especially if the testosterone decline involves photorefractoriness of the reproductive endocrine axis, as may occur in sheep at the end of breeding season (19). Thyroid hormone is required for photorefractory termination of the breeding season in sheep (28). Furthermore, prolactin has been reported to have an antigonadal effect in male rats (30).

The changes in serum concentrations of prolactin observed in male woodchucks were similar to those reported for the California ground squirrel (13), including the 4-mo elevation in late winter and early spring and the low levels throughout the summer, autumn, and early winter. Photoentrainment of the annual cycle in woodchucks and other marmotine rodents appears to require daily changes in photoperiod that simulate the natural environment, as used in the present study, and has not been produced by block changes in photoperiod (5, 10, 18, 22). Elevated prolactin secretion is associated with "long days" in both "long-day" and "short-day" seasonal breeders studied in natural daylight or during block changes in photoperiod (8). The transient elevation in prolactin observed in the spring suggests that any photoperiodic regulation of prolactin secretion in woodchucks is complex. One plausible explanation is that prolactin secretion involves a stimulation by lengthening days in early spring, a subsequent refractoriness to continued long days with a resulting decline in late spring and summer, an inhibition by shortening days in autumn and early winter, and finally a refractoriness to continuing short days with an initial increase in prolactin in the winter. Alternatively, the prolactin profile may simply occur as a component of the endogenous circannual cycle, with the endogenous cycle being photoentrained and the rate of prolactin secretion being determined by mechanisms not directly affected by photoperiod.

The late-winter and early-spring increases in prolactin observed in woodchucks in both photoperiod groups were coincident with the greatest rate of increase in relative food intake. Prolactin as well as thyroid hormone may play a role in the onset of increased food intake. Prolactin has been reported to stimulate food intake in rats (12) and in reindeer (26). In rats, central administration of prolactin causes increased food intake at doses that did not result in peripheral effects on metabolism or reproduction (27). The weight gains observed in the woodchucks during the period of increasing prolactin were modest compared with the much higher rate of weight gain observed when prolactin levels were declining. Whether or not these relationships involve a possible active role for prolactin in the regulation of lipogenesis and weight gain is not clear, because prolactin has been reported to have varied and often opposite effects on metabolism in different animal models. However, prolactin was reported to stimulate lipolysis and inhibit lipogenesis in rabbits and rats (12, 20, 24). The lipolytic effect on adipose tissue may be adaptive in female rats and favor nutrient utilization

by mammary tissue during mammary lipogenesis stimulated by lactogenic hormones (2). The antilipogenic effect in rats may involve increased adipocyte insulin resistance (24). If prolactin is likewise lipolytic or antilipogenic in woodchucks, then the weight loss and minimal weight gains observed during increasing prolactin in late winter and early spring may be related, in part, to inhibition of lipogenesis by prolactin in addition to concurrent lipolytic effects of T_4. Similarly, the subsequent maximal weight gain may be to some extent the result of the concurrent decline in prolactin and involve a derepression of lipogenesis in addition to a reduced energy expenditure caused by declining free T_4.

Prolactin at low levels has a stimulatory effect on testis function (3), but, at high levels, can be inhibitory (30). Whether or not prolactin plays either a stimulatory role at the start of the breeding season or an inhibitory role at the end of the breeding season in woodchucks remains to be studied. It is also possible that initial increases in prolactin as well as thyroid hormone play a role in termination of regulated hypothermia during winter. Prolactin prevented torpor in Siberian hamsters maintained in winterlike short-day photoperiods (25).

In conclusion, the results suggest that circannual changes in food intake, energy balance, and weight gain in photoperiod-entrained laboratory woodchucks are likely influenced, if not regulated, by seasonal changes in peripheral concentrations of free T_4 and prolactin. The extent to which the latter are controlled by photoperiod and might mediate photoentrainment of the annual cycle remains to be determined.

Perspectives

Annual cycles in mammals appear to consist of complex endogenous circannual cycles that are entrained to the solar year by environmental factors, usually photoperiod. These involve changes in multiple systems including metabolism, reproduction, integument, and neural control of food intake and other behavior. In hibernating species, these changes likely facilitate and time emergence into and emergence from seasonal hypothermia and torpor. The present results in laboratory woodchucks suggest that changes in serum-free T_4, achieved by changes in both synthesis and serum binding, and changes in serum prolactin may together orchestrate appropriately timed alterations in basal energy metabolism, fat utilization versus deposition, and relative food intake level. Woodchucks typically experience a seasonal increase in food intake of ~700% and an increase in body weight of ~40–80%, nearly all of which is fat. Whether anorectic hormones from adipocytes, such as leptin, undergo seasonal cycles and participate in physiological regulation of food intake would be of particular interest in this species.

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