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P. W. Concannon, V. D. Castracane, R. E. Rawson and B. C. Tennant

Am J Physiol Regulatory Integrative Comp Physiol, November 1, 1999; 277 (5): R1401-R1409.

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Seasonal differences in resting oxygen consumption, respiratory quotient, and free thyroxine in woodchucks

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Rawson, Richard E., Patrick W. Concannon, Paul J. Roberts, and Bud C. Tennant. Seasonal differences in resting oxygen consumption, respiratory quotient, and free thyroxine in woodchucks. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R963–R969, 1998.—The relationships among seasonal differences in body weight, food intake, metabolism, and thyroid hormone in woodchucks were investigated in 12 woodchucks. Six woodchucks had been maintained on a photoperiod simulating that found at 42°N (boreal woodchucks). The other group of six animals had been maintained similarly in all respects except that the light simulated that found at 42°S (austral woodchucks). An open-flow respirometer, calibrated using the N₂-dilution method, was used to determine metabolism twice in a 2-wk period near the September equinox, while at the same time food intake, body weight, and free thyroxine concentrations (fT₄) were measured. Body weight was the same for both groups of woodchucks. However, compared with boreal animals near their autumnal equinox, austral woodchucks near their vernal equinox had significantly higher ($P < 0.01$) daily food intake (5 ± 2 vs. 35 ± 2 g·kg⁻¹·day⁻¹), oxygen consumption (4.4 ± 0.3 vs. 7.3 ± 0.3 ml·min⁻¹·kg⁻¹), carbon dioxide production (2.8 ± 0.2 vs. 6.0 ± 0.2 ml·min⁻¹·kg⁻¹), respiratory quotient (0.65 ± 0.01 vs. 0.82 ± 0.02), and fT₄ (0.21 ± 0.01 vs. 0.65 ± 0.05 ng/dl). It was concluded that photoperiod has a strong effect on resting metabolism in the woodchuck and that there is an association between fT₄ and changes in food intake and metabolic rate.

photoperiod; thyroid hormone; food intake; body weight

LIKE MANY OF THE MARMOTINE sciurid rodents, woodchucks normally hibernate in the wild, with hibernation being initiated in the autumn, after spontaneous decreases in food intake (8, 13, 24). A period of increased food intake and of weight gain begins after emergence from hibernation in the late winter and lasts ~5 mo. Daily food intake of laboratory woodchucks fed ad libitum typically increases 300–900% during spring and summer and then decreases 75–90% during the fall and winter (5). The seasonal increases of 50–100% and decreases of 25–50% in body weight are almost entirely the result of increases and decreases in body fat content (8). These large seasonal fluctuations in body weight are presumed to be the consequence of changes in food intake and possibly metabolism (5).

It would be useful to know the contribution of changes in resting metabolism to this annual cycle of body weight. Resting metabolism was investigated in woodchucks in a study by Bailey (2) using CO₂ production (V̇CO₂) as a measure of metabolic rate. However, that study did not examine the coincident changes in body weight, respiratory quotient (RQ), or body tempera-

ture. Furthermore, it did not take into account the large changes in body weight that woodchucks undergo throughout the year (2). Because metabolism of the whole organism is proportional to body weight, it would be preferable to evaluate metabolic rate on a mass-specific basis. Food intake, body weight, and mass-specific oxygen consumption (V̇O₂) in the wild-caught yellow-bellied marmot, a related species, exposed to constant photoperiod are known to cycle on a circannual basis (27). However, concurrent determinations of related variables such as RQ and thyroid hormone concentrations have not been made. In woodchucks, an endogenous rhythm in the concentration of thyroid hormone likely affects the circannual rhythms of other physiological systems and behaviors such as food intake and metabolism.

Pronounced circannual changes in food intake, body weight, and gonadal activity have been shown in laboratory woodchucks to persist in the absence of hibernation-inducing conditions, such as photoperiod cues, food withdrawal, and lowered ambient temperature (4, 8–10, 30). The circannual cycles can be entrained by daily changes in photoperiod that simulate natural changes in photoperiod (5). It would be of interest to know whether or not cycling of resting metabolism also persists in the absence of food withdrawal and/or lowered ambient temperature.

To explore these questions, we first endeavored to establish methods for measuring resting V̇O₂ and V̇CO₂ in groups of laboratory-reared woodchucks in which the circannual cycles were 6 mo out of phase as a result of maintenance in northern vs. southern hemisphere photoperiods (5). We then examined differences in metabolism in relation to food intake, body weight, and serum concentrations of free thyroxine (fT₄). This paradigm allowed the simultaneous evaluation of “early-autumn” and “early-spring” animals under the same ambient conditions of room temperature and humidity and the same food availability, husbandry, handling, and instrumentation for measurement of metabolism. Body weight of animals maintained in the northern hemisphere photoperiod was decreasing while that of animals maintained in the southern hemisphere photoperiod was increasing (5). At the particular time of year that the present study was conducted, body weights of both groups were comparable. Despite the similarity in environmental conditions of the two groups, we hypothesized, on the basis of the marked differences in food intake but equivalent body weight at this particular time of year (5), that variables related to metabolism, including V̇O₂, RQ, and fT₄, would also be different. The results, using open-flow respirometry on animals re-

cently handled and transported to the instrumentation laboratory, demonstrated differences in resting metabolism among woodchucks in different metabolic states and indicated that metabolic rate is related to fT_4 concentration.

MATERIALS AND METHODS

Animals. Two groups of six male woodchucks each were studied. Animals were maintained in conditions in which daily photoperiod changes simulated those of either the northern hemisphere (boreal males) or the southern hemisphere (austral males). The photoperiods were designed as previously reported for animals maintained in such photoperiods for 2.5 yr (5), including animals in which the circannual cycle was phase shifted by 6 mo by transfer to austral photoperiods in June at 3 or 15 mo of age. The animals in the present study used some of these original photoentrained males (3 per group) and some of their offspring (3 per group) that had been maintained in the same photoperiods. Photoperiods used during the calendar year in the present study involved daily increases or decreases in photoperiod of 1–4 min per day and resulted in photoperiod patterns similar to those occurring naturally at 42° North and South latitudes (Fig. 1). The males were 3–7 yr old at the time of study and had been maintained in the experimental photoperiods for 3–6 yr, beginning either at birth ($n = 3$ per group), at 3 mo of age ($n = 2$ per group), or at 15 mo of age ($n = 1$ per group).

Woodchucks were fed a pelleted, hay-grain mixture ad libitum. The pellets were 89% dry matter and consisted of 15% crude protein, 2% fat, 18% crude fiber, and the remainder carbohydrate (Woodchuck Pellets, Agway, Syracuse, NY). For each animal, resting metabolism was measured twice (see below). Food intake was determined daily and averaged over 3–4 consecutive days to give an estimate of daily food intake, as previously described (5). This was done within 1 wk before each of the two measurements of metabolism. Average food intake per animal per day and per unit body weight per day was calculated for each of the two time periods.

Experimental procedures. For each animal, resting metabolism was evaluated twice: once on September 28, 1995, ± 1 day and again on October 11, 1995, ± 1 day. An additional determination of resting metabolism was made on each of

those days in two males, chosen at random, to evaluate repeatability of measurement. On evaluation days, woodchucks housed at a remote animal facility were transferred to transport cages, moved 3 miles to the laboratory, and left undisturbed for 30–180 min before testing. For each evaluation, the woodchuck's rectal temperature was recorded three times: immediately after removal from its pen, immediately before placement into the metabolism chamber, and immediately after removal from the chamber. Rectal temperatures were recorded at a distance of 7 cm into the rectum using a thermistor probe (Telethermometer model 43; Yellow Springs Instruments, Yellow Springs, OH). The metabolism chamber was constructed to permit air flow and air sampling in the absence of visual awareness by the woodchuck. The chamber was fabricated of galvanized steel stovepipe material, 8 in. in diameter, and the volume was ~ 10 liters.

Handling of the animals just before placement in the chamber usually resulted in an increased metabolic rate, which persisted for ~ 10 min. To achieve a better measure of resting metabolism, only data obtained during the last 10–20 min of each 30- to 40-min trial were used for calculation of resting metabolism. During this period, metabolic rates were generally at their lowest point and reasonably stable. Oxygen consumption and $\dot{V}CO_2$ were expressed in terms of milliliters per minute and milliliters per minute per kilogram body weight. The RQ was calculated from these values. Body weight measurements were obtained for each animal on removal from the chamber.

The instrumentation and calculations for metabolism were similar to those developed and previously used in this laboratory (15). However, air flow rate, flowmeters, and calibration procedures were adjusted to accommodate use with woodchucks. Briefly, expired gases were collected by drawing air through the chamber at a flow rate of 7 l/min. An aliquot of gas was continuously drawn from the main stream and passed through a H_2O absorber (Drierite). Oxygen and CO_2 concentrations of the sample were measured continuously (models S3-1A and CD-3A; Applied Electrochemistry, Pittsburgh, PA), and output from the analyzers was recorded, analyzed, and displayed in real time at 1-s intervals by a computer (Asyst Software Technology, Rochester, NY). Calibration of the system was accomplished using the procedures of Fedak et al. (11), in which N_2 and CO_2 were delivered via a flowmeter (model E100; Matheson Gas Products, East Rutherford, NJ). $\dot{V}O_2$ was calculated using the formula $\dot{V}O_2 = 0.26486 \times \dot{V}N_2 - [1.26486 \times \dot{V}CO_2 \times (FI_{O_2} - FE_{O_2})]$, where $\dot{V}N_2$ is the nitrogen equivalent of the fractional O_2 concentration during an experiment, FI_{O_2} is the fractional concentration of O_2 entering the chamber, and FE_{O_2} is the fractional concentration of O_2 leaving the chamber (15). This calculation yields a value for $\dot{V}O_2$ in terms of liters per minute, corrected to standard temperature and pressure. The $\dot{V}CO_2$ was determined directly based on the deflection produced by the animal of the fractional concentration of CO_2 and the equivalent calibration flow rate of CO_2 .

The flowmeter used in the calibration of the $\dot{V}O_2$ -measuring system was calibrated using a National Institute of Standards and Technology traceable precision volume meter (model 1054; Brooks Instruments, Hatfield, PA). Accuracy of the system was verified by determining the RQ for combustion of ethanol ($n = 9$) and of propane (99.2% pure, Curtin Matheson, Houston, TX; $n = 5$) inside the chamber used to measure $\dot{V}O_2$ of woodchucks. Combustion of these materials does not allow calibration of $\dot{V}O_2$ but does provide a robust validation of the accuracy of $\dot{V}O_2$ and $\dot{V}CO_2$ measurements because RQ, being a ratio, is sensitive to small changes in the measured variables (19). The RQ obtained for ethanol was

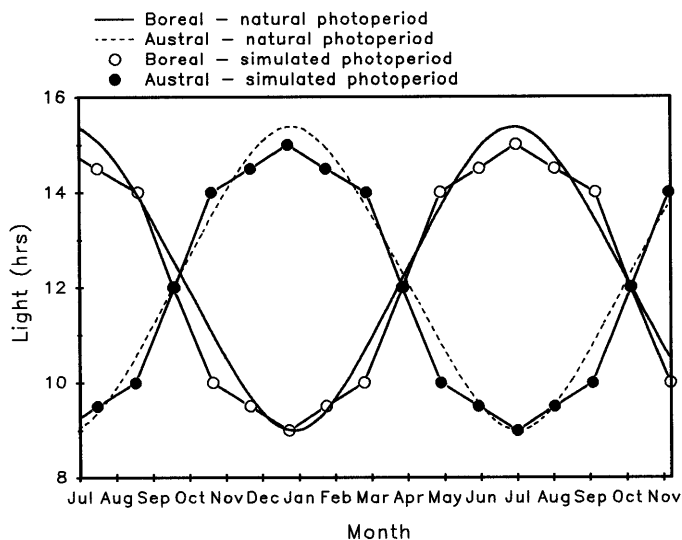


Fig. 1. Simulated photoperiods used for austral and boreal groups of woodchucks, shown in relation to natural changes in photoperiod at 42°N (boreal) and 42°S (austral) latitudes.

0.66 ± 0.02 (mean $\pm$ SD; $P > 0.05$; 95% confidence interval: 0.65–0.68; theoretical value, 0.667). The value for propane was 0.58 ± 0.02 (mean $\pm$ SD; $P > 0.05$; 95% confidence interval: 0.55–0.60; theoretical value 0.60).

Thyroid hormone. A serum sample for analysis of fT_4 was obtained 1–2 days before each measurement of metabolism. Animals were anesthetized with ketamine (50 mg/kg)-xylazine (5 mg/kg) anesthesia before collection of blood samples from the femoral vein as previously reported (5). Samples were centrifuged at 1,500 g for 20 min, and serum was stored frozen until assayed. fT_4 concentration in each of 24 samples was assayed in duplicate in a single assay using a commercial kit (DPC Coat-a-Count fT_4 ; Diagnostic Products, Los Angeles, CA). The within-assay coefficient of variation averaged 6% for all samples. The assay is a proprietary, direct, coated-tube radioimmunoassay, in which the radioactive tracer has no affinity for thyroid-binding globulin (TBG), binding to albumin is prevented by blocking agents, and an antibody that has an affinity for T_4 less than that of TBG is used at low concentrations. In both rats and woodchucks, thyroid hormone is bound to TBG. The assay used in this study has been used to measure fT_4 in rats (21). It is assumed that the assay provides relative if not quantitative estimates of differences in fT_4 in woodchucks, although results are quantitatively less than those reported when equilibrium dialysis was used to assess fT_4 in woodchucks (29). The assay was also evaluated in terms of parallelism, recovery of added hormone, and detection of physiological changes in woodchucks. Assay of serial dilutions of two woodchuck serum samples (1.22 and 0.55 ng/dl) diluted with the assay buffer yielded dilution curves for 50, 25, 12.5, 6.2, and 3.1 μ l of sample serum with slopes that did not differ ($P > 0.8$) from that of the standard curve. Recovery of standard hormone when added to the same serum samples in amounts of 1.1 and 2.3 ng/dl ranged from 71 to 89%. Assay of serum samples collected before and 6 h after thyroid-stimulating hormone (TSH; 2 μ g/kg im, Sigma) in four woodchucks demonstrated increases in fT_4 (1.1 ± 0.3 vs. 0.61 ± 0.2 ng/dl) not observed with injections of gonadotropin-releasing hormone (1 μ g/kg im).

Statistical analysis. For each variable, the two values obtained (September 28 and October 11) for each animal were averaged and the resulting value was used to calculate means for each group ($n = 6$). Means for each group are reported $\pm$ SE. Differences between groups were determined by Student's t -tests or by χ^2 tests for equality of variance (23). Differences within animals were determined by paired t -tests (23).

RESULTS

The two groups of woodchucks did not differ in body weight (Table 1). Average daily food intake in boreal males ranged from 3 to 67 g/day (mean, 18 ± 5 g/day) and was lower ($P < 0.01$) than that in austral males, which ranged from 66 to 209 g/day (mean, 131 ± 13 g/day). Food intake in relation to body weight was also lower ($P < 0.01$) in boreal than in austral males (Table 1).

Individual determinations of resting $\dot{V}O_2$ were made using data collected during periods considered to represent metabolism unaffected by acute stress of handling and placement in the chamber (Fig. 2). Resting $\dot{V}O_2$ in boreal males ranged from 3.3 to 5.9 $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ and was less ($P < 0.01$) than that of austral males, which ranged from 5.8 to 9.1 $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (Table 1). When $\dot{V}O_2$ was considered without regard to body weight, the difference between the two groups was maintained

Table 1. *Body weight, daily food intake, resting metabolism, and serum concentrations of free thyroxine near the September equinox in male woodchucks maintained in northern vs. southern hemisphere photoperiods*

	Boreal Males	Austral Males
Body weight, kg	3.9 ± 0.1	3.6 ± 0.2
Food intake, $\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$	5 ± 2	$35 \pm 2^*$
$\dot{V}O_2$, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	4.4 ± 0.3	$7.3 \pm 0.3^*$
$\dot{V}CO_2$, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	2.8 ± 0.2	$6.0 \pm 0.2^*$
Respiratory quotient	0.65 ± 0.01	$0.82 \pm 0.02^*$
Serum free thyroxine, ng/dl	0.21 ± 0.01	$0.65 \pm 0.05^*$

Values are means $\pm$ SE; $n = 6$ woodchucks in each group. $\dot{V}O_2$ and $\dot{V}CO_2$, O_2 and CO_2 consumption, respectively. *Significantly different from mean value for boreal males ($P < 0.01$).

(16.8 ± 1.1 vs. 26.6 ± 1.9 ml/min, $P < 0.01$). CO_2 production ranged from 2.0 to 4.2 $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ in boreal males and was lower ($P < 0.01$) than $\dot{V}CO_2$ of austral males, which ranged from 4.8 to 6.8 $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. The RQ ranged from 0.62 to 0.70 in boreal males and from 0.68 to 0.94 in austral males and was higher ($P < 0.01$) on average in austral males.

Serum fT_4 ranged from 0.12 to 0.26 ng/dl in boreal males and from 0.28 to 0.98 ng/dl in austral males. The mean fT_4 in austral animals was higher ($P < 0.01$) than that of boreal animals (Table 1). fT_4 was positively correlated with food intake ($r = 0.74$; $P < 0.01$), $\dot{V}O_2$ ($r = 0.68$; $P < 0.01$), and RQ ($r = 0.71$; $P < 0.01$) based on duplicate observations for each variable ($n = 24$).

Rectal temperatures before transport to the laboratory in boreal males ranged from 20.0 to 36.1°C and were more variable ($P < 0.01$) as well as lower ($P < 0.01$) than those of austral males, whose temperatures ranged from 33.3 to 38.3°C (Table 2). Rectal temperatures immediately before and after the measurement of

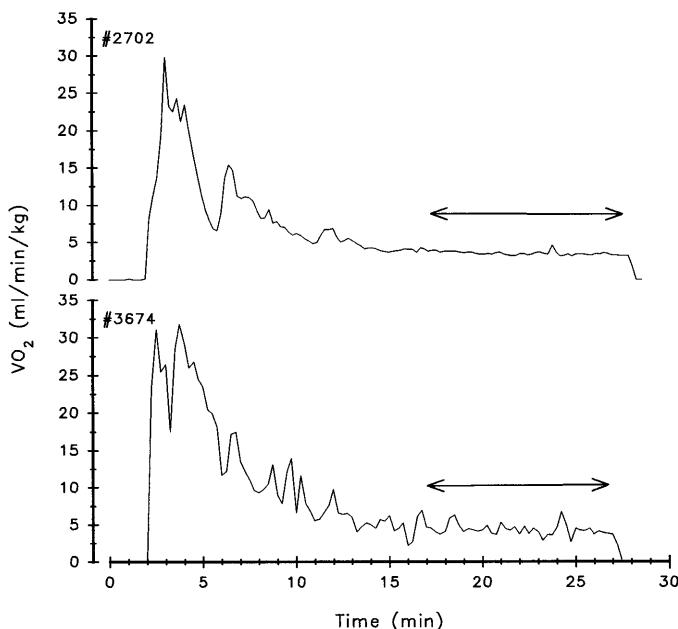


Fig. 2. Oxygen consumption ($\dot{V}O_2$) data from 2 representative boreal photoperiod, male woodchucks. Arrows indicate period over which data were used for determination of resting metabolism.

Table 2. Rectal temperatures of boreal and austral woodchucks on days that resting metabolism was measured

	Before Transport	Before $\dot{V}O_2$ Measurement	After $\dot{V}O_2$ Measurement	Change During $\dot{V}O_2$ Measurement
Sept. 28, 1995				
Boreal	28.5 ± 2.8	33.8 ± 1.3	35.5 ± 0.7	1.8 ± 0.7†
Austral	37.1 ± 0.3*	38.0 ± 0.3*	38.2 ± 0.3*	0.2 ± 0.2
Oct. 11, 1995				
Boreal	28.9 ± 2.7	32.4 ± 2.1	33.9 ± 1.4	1.5 ± 0.7
Austral	35.9 ± 0.5*	37.6 ± 0.4*	38.7 ± 0.6*	1.0 ± 0.7

Values are means ± SE in °C; $n = 6$ woodchucks in each group. *Significantly different from mean value for boreal males on the same evaluation day, $P < 0.05$. †Significantly different from 0 ($P < 0.05$).

$\dot{V}O_2$ in the metabolic chamber were also lower ($P < 0.01$) in boreal woodchucks on both experiment days (Table 2).

Repeatability of $\dot{V}O_2$ measurements was assessed by determining resting metabolism twice on two boreal and two austral woodchucks that were chosen at random. Repeated measurements of $\dot{V}O_2$ on the same woodchuck were taken ~4 h apart (Table 3). The mean decrease in $\dot{V}O_2$ of $0.31 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ over the 4-h period was significant ($P = 0.05$). Rectal temperature did not change between the two measurements for two of the woodchucks but increased by 1 and 2°C, respectively, for the other two.

DISCUSSION

These results suggest that the methods used to evaluate resting metabolism as applied in the present study are sufficiently rigorous to detect and quantitate differences in metabolic state associated with seasonal physiological changes in woodchucks.

Body weight and food intake. In this study, body weights of woodchucks housed and maintained under laboratory conditions were slightly lower than the average weight of 4.9 kg reported for wild-caught woodchucks at the same time of year (24) but are consistent with previously reported weights of laboratory-housed animals (5). Food intake values were consistent with those previously reported for animals maintained under similar photoperiods (5). Metabolic rate and food intake levels would be expected to vary according to body weight (16). Therefore, it might be postulated, because woodchucks in both groups had equivalent body weights, that metabolism and food intake would be comparable. Despite the fact that both

groups of animals had similar body weights, there was a remarkable difference in the level of food intake between the two groups of animals, which had been maintained under the same conditions except that the photoperiods were phase shifted by exactly 6 mo. The boreal animals were at their autumnal equinox, presumably in a physiological state preparatory for the period of hibernation that would occur under natural conditions. The low food intake of these animals fed ad libitum is in agreement with other reports of reduced intake during autumn in woodchucks and marmots (5, 27). The austral animals were at their vernal equinox, presumably in a physiological state approximating that which would occur following spontaneous emergence from hibernation. The high food intake in these animals is in agreement with other reports of near-peak food intake in the spring among woodchucks and marmots (5, 27). The physiological mechanisms underlying the differences in food intake between the boreal-photoperiod and the austral-photoperiod woodchucks are unclear, and further study of these phenomena is warranted, but differences in thyroid hormone status are assumed to play a role (28, 29).

Woodchuck metabolism. Mean resting $\dot{V}O_2$ measurements of $3.9\text{--}7.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ observed in woodchucks at room temperature were consistent with the value of $5.11 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ obtained for male yellow-bellied marmots (1). Bailey (2) measured CO_2 production of laboratory woodchucks and expressed his data in terms of grams per hour. In that study, mean CO_2 production for nine woodchucks in September was 1.9 g/h. In the present study, the average VCO_2 in boreal males at their autumnal equinox ($2.8 \pm 0.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$), when converted on a per animal basis into units similar to Bailey's, yielded a value of $1.3 \pm 0.1 \text{ g/h}$. These results are consistent but, because Bailey's report did not include the weight of woodchucks, a more critical comparison cannot be made. Allometric equations for metabolism relative to body size (16, 20) were used for comparison of the observed $\dot{V}O_2$ results with predicted values (Table 4). It is interesting that individual boreal-photoperiod animals at their autumnal equinox had values for $\dot{V}O_2$ that were always lower than predicted, whereas individual austral-photoperiod animals, at their vernal equinox, had $\dot{V}O_2$ values that were always higher than predicted, regardless of the predictive equation used. Armitage and Salsbury (1) also observed $\dot{V}O_2$ values that were lower than those predicted in yellow-bellied marmots, but they did not report seasonal effects.

Table 3. Repeated measures of $\dot{V}O_2$ for four woodchucks

Woodchuck	Date	First $\dot{V}O_2$ Measurement, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	Second $\dot{V}O_2$ Measurement, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	Difference, $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$	Temperature Change, °C
9072 (Austral)	Sept. 28, 1995	6.17	6.03	-0.13	0.00
9033 (Austral)	Sept. 28, 1995	5.07	4.93	-0.14	1.00
2599 (Boreal)	Oct. 11, 1995	5.21	4.79	-0.42	0.00
3674 (Boreal)	Oct. 11, 1995	4.94	4.41	-0.53	2.00
Mean ± SE		5.35 ± 0.28	5.04 ± 0.35	-0.31 ± 0.10*	

*Difference between measurements was significantly different from 0 ($P = 0.05$).



Table 4. $\dot{V}O_2$ using measured and predicted values based on allometric equations

	$\dot{V}O_2$, ml·min ⁻¹ ·kg ⁻¹		
	Measured	McNab*	Kleiber†
Sept. 28, 1995			
Boreal	3.88 ± 0.29	5.36 ± 0.07	6.96 ± 0.07
Austral	7.42 ± 0.45	5.55 ± 0.12	7.17 ± 0.13
Oct. 11, 1995			
Boreal	4.76 ± 0.39	5.41 ± 0.07	7.02 ± 0.08
Austral	7.28 ± 0.41	5.46 ± 0.12	7.07 ± 0.13

Values are means ± SE; n = 6 woodchucks in each group. *From Ref. 28. †From Ref. 16.

An important advantage of the $\dot{V}O_2$ -measuring system used in the present study is that RQ is measured rather than estimated. The RQ determinations reported here represent the first such estimates for woodchucks and the first values showing seasonal differences in any marmotine rodent. Likewise these are the first such determinations made to evaluate the seasonal changes in RQ that accompany the seasonal changes in body weight and body fat content (8). The finding of lower RQ values in autumnal equinox boreal males and higher RQ values in vernal equinox austral males is consistent with the austral animal's having a carbohydrate-rich diet and high food intake level and the boreal animal's depending almost entirely on fat stores in the face of negligible food intake. Values for RQ of ~0.7 have been reported for marmots during the winter (25).

Repeatability of $\dot{V}O_2$ measurements. Several hours elapsed between $\dot{V}O_2$ measurement of the first and the last woodchucks on a given day. We were therefore interested in the variability in $\dot{V}O_2$ introduced as a consequence of transport and confinement in a cage inside the laboratory for variable lengths of time. Variability could stem from changes in thermogenic activity as a result of activation of the sympathetic nervous system during transport. An animal placed in the metabolic chamber soon after arriving in the laboratory might have a $\dot{V}O_2$ that was increased simply as a consequence of transport stress.

In four woodchucks measured twice on the same day, there was a significant ($P = 0.05$) and consistent decline in $\dot{V}O_2$ as woodchucks "rested" in the laboratory. However, $\dot{V}O_2$ declined by an average of only 6% (0.31 ml·min⁻¹·kg⁻¹). It was unlikely that this small decline was related to changes in body temperature because rectal temperature of the woodchucks either did not change or increased. A significant increase in body temperature would more than likely have been associated with an increase, rather than a decrease, in metabolic rate. The consistent decline in $\dot{V}O_2$ suggested that transport may have stimulated the sympathetic nervous system sufficiently to elevate $\dot{V}O_2$. Nevertheless, the effect of resting in the laboratory was a very small one relative to the large difference in $\dot{V}O_2$ between the austral and boreal woodchucks.

Body temperature and $\dot{V}O_2$. The average increase in rectal temperature of 6°C in boreal animals overall,

from before transport until removal from the metabolic chamber, was significantly greater ($P < 0.01$) than the increase of 2°C in austral woodchucks. However, most of the increase in body temperature occurred before entrance into the metabolism chamber because there was no significant increase in temperature during $\dot{V}O_2$ measurement. Consequently, our measurements of $\dot{V}O_2$ represented resting metabolism and were not confounded by acute increases in body temperature.

The lower rate of heat production in the boreal animals undoubtedly contributed to the reduced temperatures observed in these animals, compared with those in austral animals. At the same time, a lower body temperature can result in a slower rate of metabolism. Oxygen consumption of the boreal woodchucks was 40% lower than that of the austral woodchucks, and mean rectal temperature of the boreal woodchucks was always lower than that of austral woodchucks. Because the rate of cellular metabolism is highly temperature dependent, it is not surprising that those individuals with the lowest rectal temperatures, that is, the boreal woodchucks, would have the lowest metabolic rates.

However, temperature is not the only influence on metabolic rate. First, thyroid hormones are known to increase basal metabolism (14). In the present study, higher fT_4 concentrations in the austral woodchucks correlated with the higher rates of metabolism in these animals relative to the boreal woodchucks. Second, thermal insulation influences metabolic rate via a relationship analogous to Ohm's law. For a given body temperature-to-ambient temperature gradient, an increase in thermal insulation would allow a reduction in metabolic rate. The boreal woodchucks could have increased their thermal insulation, via changes in hair coat or fat distribution to subcutaneous sites. Indeed, Armitage and Salsbury (1) have presented preliminary evidence that body temperature in yellow-bellied marmots does not necessarily decline along with $\dot{V}O_2$, indicating that changes to insulation may be a primary strategy for conserving energy while at the same time maintaining body temperature at some thermoregulatory set point. A third alternative explanation for the reduction in metabolic rate of the boreal woodchucks is that a decrease in body temperature reduces the core-to-ambient temperature gradient and, therefore, the driving force (the core-to-ambient temperature gradient) for heat flow from the body to the environment is also diminished. This would allow for metabolic rate to be reduced. Thus the lower metabolic rate observed in boreal woodchucks is likely multifactorial and presumably involves a withdrawal of thyroid hormones and a decrease in the body temperature but may also involve an increase in thermal insulation.

A comparison of the standard errors associated with rectal temperatures indicates that boreal woodchucks at their autumnal equinox were more thermolabile than austral woodchucks at their vernal equinox. It is likely that thermolability in the woodchuck is an energy-conservation strategy in which a low body temperature minimizes heat loss by reducing the core-to-

ambient temperature gradient. The low temperatures observed among boreal woodchucks likely correspond to the even lower temperatures observed in natural hibernation. Presumably then, body temperature of boreal woodchucks near their autumnal equinox rises only in association with activity- or stress-related increases in $\dot{V}O_2$. It remains to be determined, however, whether changes in body temperature of boreal woodchucks are controlled via an altered set point or whether temperature rises passively as a consequence of increases in $\dot{V}O_2$ or changes in insulation. Additional studies will be required to assess the relative roles of heat production, heat loss, and insulation in the changes in body temperature of the woodchuck.

Influence of thyroid hormone on metabolic status. Thyroid hormone status was estimated in this study by measurement of fT_4 because there are seasonal changes in TBG in woodchucks (29). Such changes result in large seasonal differences in total thyroxine levels and make measurement of total hormone an unreliable measure of peripheral thyroid hormone status. The mean concentrations of fT_4 (0.21–0.65 ng/dl) observed in this study were similar to those in a preliminary report of fT_4 in woodchucks maintained in similar photoperiods and using the same assay method (6) but were lower than the concentration of 1–3 ng/dl measured in woodchucks by an equilibrium dialysis method (30). The results of radioimmunoassay used to measure fT_4 can be affected by differences between species in the affinity of binding proteins relative to the affinity of the antibody employed and in the ability of the assay matrix components to stabilize endogenous binding. Although the absolute values observed for woodchucks may not be quantitatively accurate, the apparent validity of the assay based on parallelism and recovery of added hormone leads us to assume that the relative differences observed are real. The results show that fT_4 levels at the vernal equinox in austral animals were three times those at the autumnal equinox in boreal animals. The ~200% differences between groups in mean fT_4 observed in this study were not unexpected. In the present study, serum fT_4 was low in boreal animals at the autumnal equinox, whereas previous studies (28, 29) showed that TBG was highest and total serum T_4 was increased at that time. Evidently, changes in TBG may determine the availability of T_4 . The observation that TBG binding of T_4 is increased in autumn and winter when the thyroid gland appears inactive and decreased in spring and summer when the thyroid gland appears active would lead to a prediction of higher levels of fT_4 in the spring than in the autumn (29). The present findings validate such a prediction.

Thyroid hormone is the primary endocrine regulator of obligatory thermogenesis, that component of total thermogenesis arising from essential cellular metabolic reactions (14). The metabolic effects of thyroid hormone are mediated by effects on mitochondrial number and function, as well as sensitivity to catecholamines, among others (12). In rats, adaptation to a cold environment or stress will increase thyroid hormone via an increase in TSH secretion (14). In the present study, the two groups

of woodchucks were housed in a laboratory setting under similar environmental conditions except for photoperiod. It is likely, therefore, that differences between groups in thyroid hormone concentration were the consequence of an endogenous photoperiod-entrained rhythm rather than thermal or other environmental influences. The austral-photoperiod animals, along with having a higher metabolic rate, also had a higher thyroid hormone concentration than boreal woodchucks. Because $\dot{V}O_2$ was measured under the same conditions in both groups of animals, it is likely that the increased thyroid hormone concentration in the austral animals played a role in the increase of metabolic rate.

The cause-and-effect relationships among several of the measured parameters are not entirely clear. However, the higher resting metabolism, food intake, and RQ in the austral animals compared with the boreal animals was very likely associated with the difference in thyroid state. That fT_4 was significantly correlated with food intake, $\dot{V}O_2$, and RQ supports this notion.

The higher concentrations of T_4 in the austral (vernal equinox) animals would be expected to increase resting metabolism as a result of the direct calorogenic effect of T_4 (12) and to increase food intake just as in thyrotoxicosis (18). The increased RQ would be expected from the increased food intake and decreased dependence on fat stores, because the diet is primarily carbohydrate and protein. Similarly, the lower concentrations of T_4 in the boreal (autumnal equinox) animals would be expected to lower resting metabolism and reduce food intake, as observed in hypothyroidism (18). Decreased dietary caloric intake would be expected to result in increased metabolism of fat and a reduced RQ.

The experimental design of the present study involved establishment of two groups of woodchucks for which photoperiod was the only distinguishing treatment. The woodchucks were fed ad libitum, and the decreased food intake by boreal animals was strictly voluntary. This suggests that a decline in food availability, such as might occur in the wild, is not a prerequisite for seasonal decreases in metabolic rate. Metabolic state could also have been dependent on some critical body size, but in the present study, woodchucks in both groups weighed the same. It would be reasonable, therefore, to hypothesize that photoperiod, rather than other factors, regulates the timing of the seasonal changes in fT_4 , presumably via changes in TBG production (30) and TSH secretion (26).

In addition to T_4 , the autonomic nervous system and catecholamines are likely to be involved in the control of food intake and metabolism of these animals (17). The increased food intake itself would be expected to contribute to the increased resting metabolism (22) and increased thyroid hormone concentrations (7). Seasonal changes in food intake are probably also regulated by body fat content, and the reduced food intake in the boreal animals could reflect effects of leptin or a comparable regulatory peptide from adipose tissue as demonstrated in mice (3).

In conclusion, the present study measured metabolism to evaluate differences between male laboratory

woodchucks in different periods of their circannual cycle. Woodchucks maintained in a boreal photoperiod that simulated that found at 42°N latitude were experiencing shortening days. The woodchucks in the austral photoperiod that simulated the light cycle at 42°S were experiencing lengthening days. Despite photoperiod being the only difference in the maintenance of these animals, the contrasts between the two groups were striking. Food intake, VO_2 , VCO_2 , RQ, and fT_4 of austral woodchucks that were near their vernal equinox were all higher than the same variables in the boreal woodchucks that were near their autumnal equinox. Furthermore, food intake, VO_2 , and RQ were significantly correlated with fT_4 . While it is likely that thyroid hormone concentrations contributed to the physiological differences observed in these woodchucks, further study will be required to characterize and expand our understanding of the processes underlying the circannual rhythms in energy metabolism.

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