

Thyroid Hormone Concentrations in Black Bears (*Ursus americanus*): Hibernation and Pregnancy Effects

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Previous studies on thyroid hormones in hibernating bears have used very few sampling periods, so that the time course of any change is poorly understood. In this study, plasma sampled from pregnant and nonpregnant black bears before and during hibernation (16 samples each at 10-day intervals) was assayed by radioimmunoassay for concentrations of thyroxine (T_4) and triiodothyronine (T_3). Only free T_4 showed a difference ($P = 0.019$) between females that produced cubs and those that did not, but this appeared to be due to higher preimplantation values. Free T_3 , total T_3 , and free T_4 varied ($P = 0.001, 0.038, 0.002$, respectively) among sampling periods: during December, bears had depressed concentrations. These lowered concentrations were maintained during hibernation for the free hormones. Our data confirm previous work showing that food restriction and/or physiological preparation for hibernation is coincident with depressed plasma concentrations of thyroid hormones. Hormonal changes associated with pregnancy were minor. © 1998 Academic Press

Black bears (*Ursus americanus*) hibernate 4–7 months (Nelson *et al.*, 1983), and during this time, females implant blastocysts, give birth to cubs, and lactate. The breeding season of the black bear usually occurs from May to July (Garshelis and Hellgren, 1994; Mead, 1989), with the delayed implantation of blastocysts occurring in December (Wimsatt, 1963). Typically in

autumn, they reduce their feeding and then enter hibernation. Parturition occurs about 60 days after implantation, in January or February (Foresman and Daniel, 1983; Hellgren *et al.*, 1991). Lactation and hibernation continue together for 10–12 weeks postpartum, until mid to late April.

Whether the winter dormancy that bears undergo is a form of true hibernation has been debated, but it is clearly not the “deep” hibernation seen in smaller hibernators, where body temperature can drop by 30°C or more and metabolic rate can drop by 90%. While there are some important differences, bear hibernation shares many basic physiological characteristics with that of “deep hibernators.” The relatively small drop in the body temperature of hibernating bears (<10°C) is thought to be due to their large mass (Geiser and Ruf, 1995; Heldmaier *et al.*, 1993), rather than a fundamental physiological distinction. That bears complete gestation during this hibernation (and associated low metabolism) is unusual, but this also may be mass-related. Although bears have short implantation to parturition periods for their size, they would not have time to produce successful litters before the next winter if they waited until hibernation was terminated in order to breed. A bear fetus is born during hibernation in a very altricial state due to constraints on hibernation metabolism and fetal fuel requirements (Ramsay and Dunbrack, 1986).

In humans, thyrotropin (thyroid-stimulating hormone) gradually increases during the second and third trimesters (Burrow, 1993). Increases are also seen in serum concentrations of thyroxine-binding globulin, total triiodothyronine (T_3), and total thyroxine (T_4). However, concentrations of free hormones and measures of thyroid function such as turnover and clearance are relatively unaffected or may decline (Burrow, 1993).

Other species are not as well studied, but pregnancy has been associated with a decrease in total T_4 in hamsters (*Mesocricetus auratus*: Galton and Galton, 1966), a decrease in total T_3 (Flisinska-Bojanowska *et al.*, 1991) but no change in protein-bound iodine (PBI) (Irvine, 1967) in horses, an increase in PBI in rhesus monkeys (*Macaca mulatta*: Stolte *et al.*, 1966), a decrease in PBI in early-pregnancy lab rats (Feldman, 1958), an increase in PBI in late-pregnancy lab mice (Wills and Schindler, 1970), and a decrease in total T_4 and total T_3 in late-pregnancy rats (Calvo *et al.*, 1990). Some of these differences may be species-specific, and others may relate to different times during the pregnancy being studied. Unfortunately, no data exist for thyroid hormone concentrations in pregnant black bears.

The effect of hibernation on thyroid function is still unresolved. In ground squirrels (*Spermophilus*), Bauman and Anderson (1970) found no change in serum PBI, but T_4 utilization rate (disappearance of hormone per day) virtually stopped. By contrast, Demeneix and Henderson (1978) reported no change in T_4 utilization, but a 5- to 10-fold increase in serum T_4 , free T_4 , and T_3 during hibernation. Magnus and Henderson (1988a,b) also reported increases in free and total T_4 and T_3 during hibernation. They attributed the increase in total hormones to an increased binding to plasma proteins (Magnus and Henderson, 1988a) and the increase in free hormones to a decrease in number of intracellular T_3 receptors (Magnus and Henderson, 1988b). In some studies, thyroid depression was pronounced in the fattening period prior to hibernation (Hulbert and Hudson, 1976; Tomasi and Stribling, 1996).

In black and Himalayan bears (nonpregnant) before hibernation, T_3 was cleared more rapidly from the circulation than T_4 , but clearances were similar during hibernation (Nelson *et al.*, 1973). Examination of a thyroid gland in a bear after 2 months of hibernation

revealed normal histology (Nelson *et al.*, 1973). However, early studies of temporal changes in serum concentrations of thyroid hormones reported decreases with hibernation (Lundberg *et al.*, 1976) or no change (Nelson *et al.*, 1973). The most complete study on thyroid physiology in black bears (Azizi *et al.*, 1979) showed significant declines in total and free concentrations of both T_4 and T_3 from October through March (monthly samples), with increasing posthibernation concentrations in April and May. These endocrine changes were supported by Hissa *et al.* (1994), who reported decreases of approximately 50% in total and free T_3 and T_4 in a dormant Finnish brown bear.

Baseline concentrations of thyrotropin in dormant and active states were similar (Azizi *et al.*, 1979). However, the thyrotropin response to thyrotropin-releasing hormone (TRH) during hibernation was prolonged and exaggerated relative to that during the posthibernation period. Serum T_3 and T_4 also increased following a TRH challenge, although for T_3 , the increase was greater during hibernation. These data led Azizi *et al.* (1979) to propose that bears have a hypothalamic hypothyroidism during hibernation. That is, the thyroid gland itself appears to be normal during hibernation, but its function is reduced because of a deficiency of TRH. This is consistent with an observation in ground squirrels that the thyroidal content of iodine and thyroglobulin was not changed by hibernation (Demeneix *et al.*, 1983).

In bears, voluntary decreases in food consumption occur simultaneously with, and sometimes precede, hibernation. Most studies reviewed by Tomasi (1991) found that food restriction reduced T_4 utilization rate (12–33%), T_3 utilization rate (50%), and concentrations of serum T_3 (50%), but did not change serum T_4 .

Our objective was to describe the patterns of total and free T_3 and T_4 serum concentrations in female black bears during the time of the potentially interacting effects of pregnancy and hibernation. This is the first study to directly examine the effects of pregnancy on these parameters. We predicted that the free T_3 and T_4 concentrations would decline during hibernation, regardless of pregnancy. Conversely, we predicted that the thyroidogenic effect of gestation may partially offset the hibernation-associated decrease in total hormone concentrations.

METHODS

Female bears, either captive-reared ($n = 2$) or wild-caught after August 1987 ($n = 4$) and August 1988 ($n = 5$), were maintained in captivity (separate pens in an open-sided barn) but exposed to ambient temperature and photoperiod at Center Woods Research Facility, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Six bears were maintained each year (one captive-reared bear was used during both years of the study). All were adults (≥ 3 years old) and had opportunities for mating prior to inclusion in the study. Each year, one bear did not hibernate, and data from these are not included in the analyses.

All bears were fed 1.0 kg of commercial omnivore chow daily until 30 September. From 1 October to 1 December, more food (2.0 kg in 1987, 3.0 kg in 1988) was provided daily to allow bears to increase body mass. During December, daily rations were reduced by 50% every 10 days to simulate field conditions, and feeding frequency was reduced to alternate days (2 days of rations at a time) to minimize disturbance. If bears did not consume all food offered on two separate occasions, food was no longer provided. All feeding was discontinued on 3 January. Water was provided throughout the experiment. Onset of hibernation (as confirmed by lethargy, a small decrease in body temperature, nest construction and extended occupancy, and a marked decrease in serum urea/creatinine ratios; Hellgren *et al.*, 1990) varied between bears, but occurred between 4 December and 10 January. Mean body mass at this point was about 85 and 97 kg for the 2 years. Hibernation lasted an average of 92 days. Detailed methods for bear maintenance and blood collection were described in Hellgren *et al.* (1990, 1991); the data provided herein represent a further analysis of the same blood samples used in these two earlier studies.

Bears were immobilized with ketamine hydrochloride (6.6 mg/kg; not known to affect thyroid hormone concentration) at approximately 10-day intervals from 30 September to 31 March, and blood samples were collected from a femoral vein with heparinized vacuum tubes 11.4 ± 0.5 min after immobilization (mean \pm SE). When the mass of a bear decreased to its level from the previous summer, food was provided and the bear terminated hibernation. Subsequent blood samples were not used in this study. Blood was centrifuged at

1450g for 15–20 min within 2 h after collection. Plasma was separated and stored frozen at -20°C (4–6 years) until shipped to Southwest Missouri State University.

Thyroid hormones were assayed in duplicate using radioimmunoassay kits (INCSTAR Clinical Assays, Stillwater, MN), specifically GammaCoat [^{125}I] Total T_3 , GammaCoat [^{125}I] Total T_4 , GammaCoat [^{125}I] Free T_3 , and GammaCoat [^{125}I] Free T_4 . To avoid interassay variation, all samples were measured in one assay per hormone measurement. Procedures for each assay followed those provided with the kit, with the following modifications: plasma was used instead of serum; the plasma volumes were doubled and the obtained values were then halved; and different standard concentrations were used (total T_3 , 0.25–5.0 ng/ml; total T_4 , 5–80 ng/ml; free T_3 , 0.425–36.3 pg/ml; and free T_4 , 0.9–42 pg/ml).

The intra-assay variation (mean of the coefficients of variation for triplicates) for the standards of each kit were total T_3 , 1.24%; total T_4 , 1.56%; free T_3 , 5.04%; and free T_4 , 8.72%. Each radioimmunoassay was validated for black bears by yielding expected patterns when the plasma pool (a combination of various black bear plasma) was diluted to 25 and 50% with serum blank from the kits (not really zero), doubled, tripled, and mixed (50:50) with known concentrations of the respective hormone (standards from each RIA kit). The mean difference between observed and calculated values were total T_3 , +3%; total T_4 , +21%; free T_3 , +48%; and free T_4 , -0.3%.

Bears were divided into two groups: hibernating pregnant ($n = 5$) if they gave birth to young during hibernation, or hibernating nonpregnant ($n = 5$) if no cub was produced. Hormone concentration data were then statistically analyzed with a two-way ANOVA (General Linear Model, Minitab 11.2 for Windows) to determine time and pregnancy effects. The effect of pregnancy was also examined independently (t test), and the time effect was examined separately for pregnant and nonpregnant bears (one-way ANOVA). Free/total hormone ratios and T_3/T_4 ratios were also calculated from these data and analyzed in the same manner. The latter ratios were mass/mass ratios and were not adjusted for molecular weight.

Because these tests assume independence of the data and do not take into account interanimal variation, it is more difficult to obtain significant differences

($P < 0.05$). Therefore, the data were also analyzed with repeated-measures and multivariate two-way ANOVA (SAS, version 6, 1990) after the study period was reduced to three intervals (due to software limitations prohibiting the use of more sampling times than there were bears) and the average value for each bear in each time interval was calculated. These time intervals were prehibernation with *ad lib.* feeding, prehibernation with reduced food, and hibernation. On two occasions, a nonpregnant bear hibernated spontaneously, before blood samples were taken in the reduced food interval, and the statistical program would not accept these animal-years in the statistical analyses (i.e., sample size for statistical analysis was 3 for nonhibernating bears).

RESULTS

For all bears combined, total T_3 , free T_3 , and free T_4 significantly differed among time periods (two-way ANOVA: $P = 0.001, 0.038, \text{ and } 0.002$, respectively). For all hormones except total T_3 in nonpregnant bears, concentrations tended to drop during December, the time of prehibernation food restriction, onset of postimplantation pregnancy, and entry into hibernation. Free hormone concentrations remained more or less depressed through hibernation, especially free T_4 in pregnant bears (Fig. 1); total hormone concentrations tended to return to prehibernation concentrations after

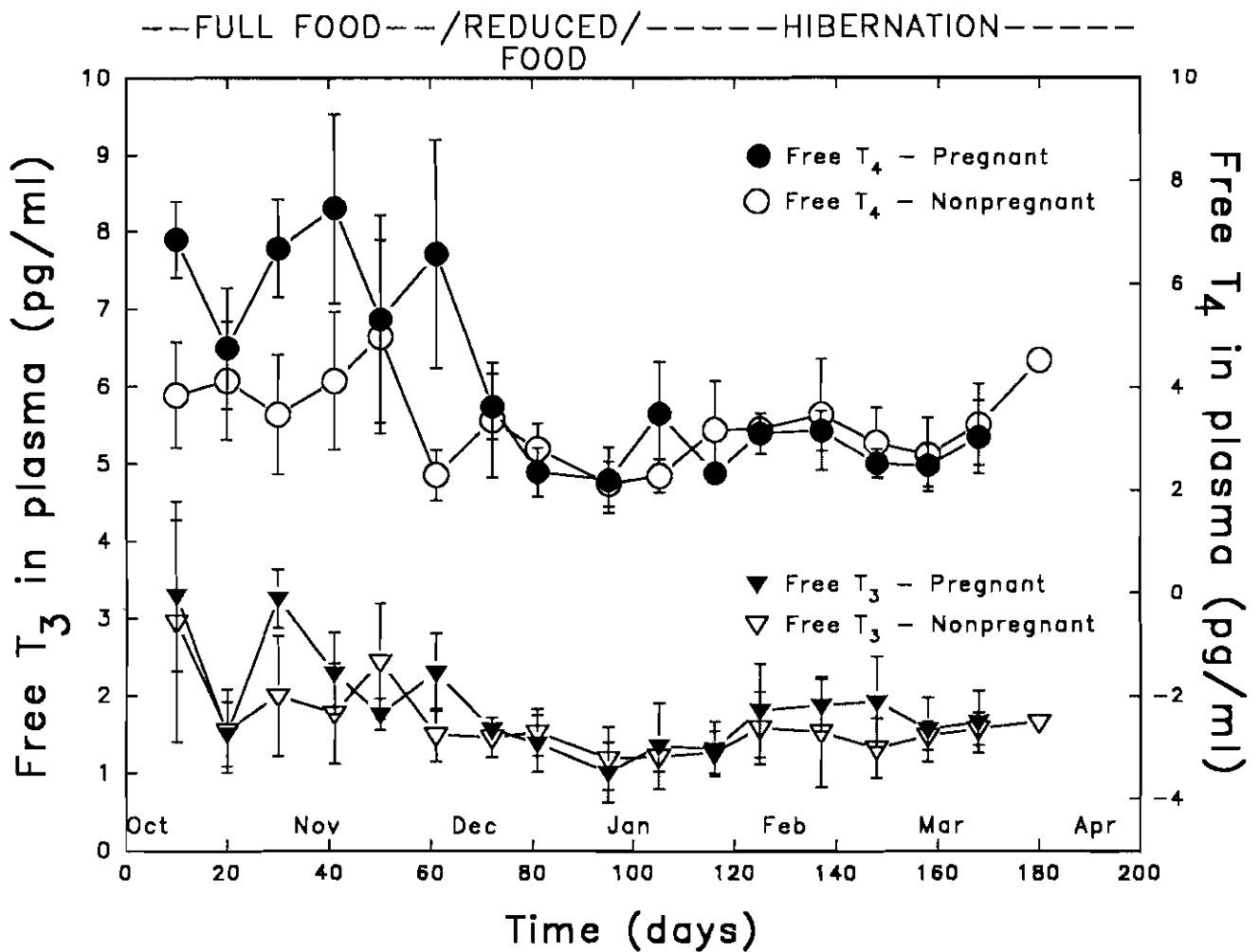


FIG. 1. Concentrations (mean \pm 1 SE; $n = 5$) of free T_4 (circles and the right axis) and free T_3 (triangles and the left axis) in pregnant (closed symbols) and nonpregnant (open symbols) black bears in captivity (Blacksburg, Virginia: 1987–1988 and 1988–1989) prior to and during hibernation. Approximate times for the three time intervals identified in the text are shown at the top.

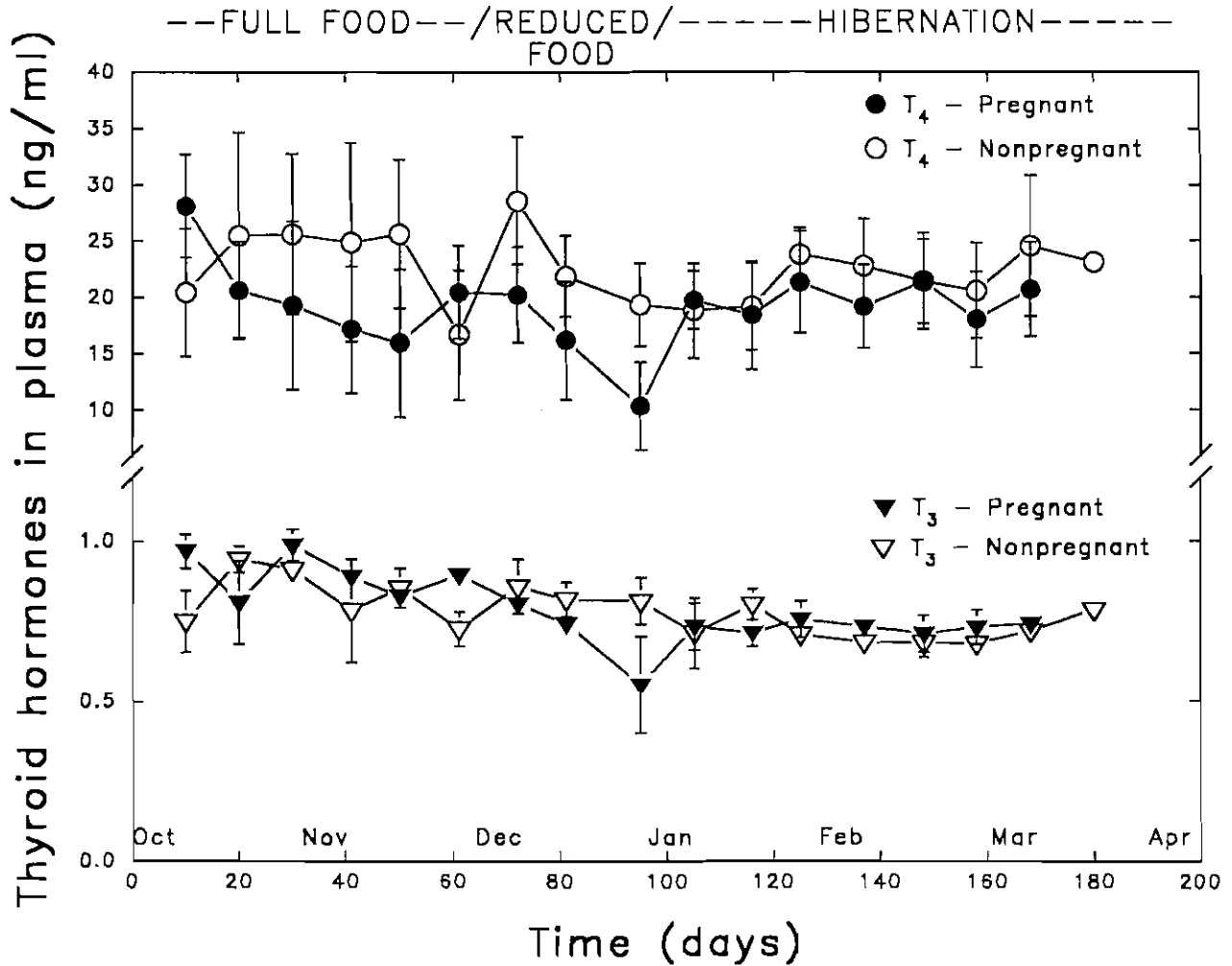


FIG. 2. Concentrations (mean \pm 1 SE; $n = 5$) of total T₄ (circles) and total T₃ (triangles) in pregnant (closed symbols) and nonpregnant (open symbols) black bears in captivity (Blacksburg, Virginia: 1987–1988 and 1988–1989) prior to and during hibernation. Approximate times for the three time intervals identified in the text are shown at the top.

1 January (Fig. 2). Separate time analyses for the bears that produced cubs (one-way ANOVA) led to statistical inferences similar to those seen for the "all bears" data (two-way ANOVA), but no hormone varied ($P > 0.05$) over time for nonpregnant bears. The repeated-measures analyses of the compressed data set for all bears yielded the same conclusion as obtained with the nonrepeated-measures analyses using the complete data set: total T₃, free T₃, and free T₄ were affected by time (two-way ANOVA: $P = 0.022$, 0.010 , and 0.011 , respectively).

Only free T₄ showed differences associated with pregnancy (two-way ANOVA: $P = 0.019$), and this

difference appeared to be primarily in the earlier sampling periods (Fig. 1). Although total T₄ mean values were lower in pregnant than in nonpregnant bears for most collection dates (Fig. 2), this was not significant ($P = 0.076$). There were no significant interaction effects between time and pregnancy (two-way ANOVA). Direct comparisons of values from pregnant and nonpregnant females also showed that only free T₄ was significantly different (t test; $P = 0.026$). With the repeated-measures analyses of the compressed data set, pregnancy did not affect any of the hormone concentrations.

The ratios of T₃/T₄ and free/total T₃ showed no

effect of pregnancy, time, or their interaction (data not shown). The free/total T_4 ratios also showed no effect of time, but were higher in bears which produced cubs ($P = 0.043$). Using the repeated-measures analyses and the compressed data set, there were no effects on the T_3/T_4 ratios or the free/total T_4 ratios; the free/total T_3 ratios decreased with time ($P = 0.005$).

DISCUSSION

Despite the fact that the plasma used in this study was frozen for up to 6 years, and various procedures have been used to measure serum concentrations in different studies, the values presented herein are within the ranges seen in previous studies of thyroid hormones in bears (Azizi *et al.*, 1979; Hissa *et al.*, 1994; Nelson *et al.*, 1973). However, the frequent sampling in this study provides a more complete chronology than previously possible. The free/total hormone ratios are also unremarkable; these would be much higher if carrier proteins had been degraded over the period of frozen storage. Unfortunately, hormone concentrations (each of which represents a single point in time) do not tell the entire story. Dynamic measures such as turnover, clearance, or deiodination rates are needed for unambiguous interpretation of the data. Nonetheless, hormone concentrations contribute to an overall model of thyroid function and to our understanding of how physiological and ecological factors such as hibernation or pregnancy affect this model.

The most obvious perturbation to concentrations of thyroid hormone occurred in December, when concentrations generally decreased, regardless of reproductive state. This timing coincided with the onset of postimplantation pregnancy (Hellgren *et al.*, 1991). However, because decreases were also seen in nonpregnant bears, pregnancy did not appear to have a strong effect on these parameters. Only free T_4 and the free/total T_4 ratio, differed based on reproductive status (at the $P < 0.05$ level), but most of this difference occurred before implantation. While it is possible that higher preimplantation levels indicate some higher quality of health, such that the probability of successful reproduction would be improved, the biological significance of this finding is unclear.

Hellgren *et al.* (1991) suggest, based upon early

ultrasound examination, that fetal resorption may have occurred in two of the "nonpregnant" bears in this study. Because progesterone values and pattern did not differ between the two groups of bears included in our study, these reabsorptions cannot be verified. Pseudopregnancy and/or implantation failure could have occurred in the others, and these may have contributed to the lack of a pregnancy effect in other hormone measurements. Alternatively, bear neonates are extremely altricial and have only a fraction of the mass predicted by allometric relationships (Ramsay and Dunbrack, 1986). Therefore, gestational adjustments in thyroid function for metabolic purposes may be small as well.

December is also the time of final physiological preparations for hibernation. Our data suggest that physiological changes associated with onset of hibernation include a general drop in concentrations of thyroid hormones and are consistent with the most intensive prior study on bears (Azizi *et al.*, 1979) and with the majority of studies on other species during hibernation (Tomasi, 1991; Tomasi and Stribling, 1996). In addition to the 10 bears described above, 2 others had been included in the study, but did not hibernate. Their levels of total T_3 and total T_4 stayed fairly stable throughout the study, at prehibernation levels; free T_3 and free T_4 appeared to increase just prior to hibernation or toward the end of hibernation, respectively. Although the sample size is inadequate for analysis, these observations support the conclusion that a drop in thyroid hormone concentrations is associated with hibernation, rather than just a seasonal change. A prehibernation decrease in thyroid function might facilitate fat deposition (Gorbman *et al.*, 1983) and/or increase membrane fluidity at lower body temperatures (Hulbert, 1978). The fact that free hormone concentrations remained relatively low for the duration of hibernation suggests that this change is associated with hibernation as well as the prehibernation period.

The prehibernation period coincides with a reduction in food availability, both naturally and in the present study. This makes it difficult to separate the effects of food reduction and prehibernation. Because both states have been previously associated with a depression of thyroid function (Hulbert and Hudson, 1976; Tomasi, 1991; Tomasi and Stribling, 1996), it is not

possible to assign relative contributions of these two factors to the observed decrease.

Changes in plasma-binding proteins can be deduced from a comparison of concentrations of free and total hormones. The free/total hormone ratios did not change significantly over time. When combined with the initial decline in concentrations of free hormones, these data indicate that the initial decline in total hormones was not merely a result of a decline in binding proteins. However, the subsequent increase in concentrations of total hormones, but not free hormones, suggests that an increase in binding proteins is occurring. This is supported by the significant decrease with time in the free/total T_3 ratio (two-way ANOVA: repeated-measures analyses of compressed data).

The apparent increase in thyroid-binding proteins could be due to pregnancy (Burrow, 1993). It was most pronounced in the T_4 data from the pregnant bears, although the effect of pregnancy was not statistically significant ($P = 0.076$) in our analysis. An increase in binding proteins could also be due to hibernation, as seen in ground squirrels (Magnus and Henderson, 1988a), and is consistent with hibernation-induced changes in protein metabolism reported in black bear (Lundberg *et al.*, 1976). After protein degradation, nitrogen is incorporated into plasma proteins faster than it is entered into the urea cycle. The net result is a decrease in blood urea and an increase in serum albumin and total protein (Nelson *et al.*, 1983; Hellgren *et al.*, 1990). Because bears have no prealbumin (Nelson *et al.*, 1973), concentrations or activity of binding proteins presumably relate to thyroid-binding globulin and albumin. Analysis of serum chemistry from the same blood samples used in the present study revealed increases in serum concentrations of globulin, albumin, and total protein in hibernators (Hellgren *et al.*, 1990). Changes in these variables were not affected by pregnancy, although globulin showed a significant interaction between reproductive state and time (Hellgren *et al.*, 1990).

In summary, this paper demonstrates that pregnancy in black bears does not noticeably influence thyroid hormone concentrations. This was not unexpected, as the preparturition reproductive cost is relatively small in bears. The prehibernation period, a time of intense metabolic changes, was associated with depressed thyroid hormone concentrations.

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REFERENCES

- Azizi, F., Mannix, J. E., Howard, D., and Nelson, R. A. (1979). Effect of winter sleep on pituitary-thyroid axis in the American black bear. *Am. J. Physiol.* **237**, 227-230.
- Bauman, T. R., and Anderson, R. R. (1970). Thyroid activity of the ground squirrel (*Citellus tridecemlineatus*) using a cannula technique. *Gen. Comp. Endocrinol.* **15**, 369-373.
- Burrow, G. N. (1993). Thyroid function and hyperfunction during gestation. *Endocr. Rev.* **14**, 194-202.
- Calvo, R., Obregon, M. J., DeOna, C. R., Ferreiro, B., Del Rey, F. E., and De Escobar, G. M. (1990). Thyroid hormone economy in pregnant rats near term: A "physiological" animal model of nonthyroidal illness? *Endocrinology* **127**, 10-16.
- Demeneix, B. A., and Henderson, N. E. (1978). Thyroxine metabolism in active and torpid ground squirrels, *Spermophilus richardsoni*. *Gen. Comp. Endocrinol.* **35**, 86-92.
- Demeneix, B. A., Lachiver, F., and Henderson, N. E. (1983). Thyroidal iodine and thyroglobulin in hibernating and active Richardson's ground squirrel *Spermophilus richardsoni*. *Comp. Biochem. Physiol. A* **75**, 273-284.
- Feldman, J. D. (1958). Iodine metabolism in pregnancy. *Am. J. Physiol.* **192**, 273-278.
- Flisinska-Bojanowska, A., Komosa, M., and Gill, J. (1991). Influence of pregnancy on diurnal and seasonal changes in cortisol, T_3 and T_4 levels in mare blood serum. *Comp. Biochem. Physiol. A* **98**, 23-30.
- Foresman, K. R., and Daniel, J. C., Jr. (1983). Plasma progesterone concentrations in pregnant and nonpregnant black bears (*Ursus americanus*). *J. Reprod. Fertil.* **68**, 235-239.
- Galton, V. A., and Galton, M. (1966). Thyroid hormone metabolism in the pregnant hamster, *Mesocricetus auratus*. *Acta Endocrinol.* **53**, 130-138.
- Garshelis, D. L., and Hellgren, E. C. (1994). Variation in reproductive biology of male black bears. *J. Mammal.* **75**, 175-188.
- Geiser, F., and Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds: Physiological variables and classification of torpor patterns. *Physiol. Zool.* **68**, 935-966.
- Gorbman, A., Dickoff, W. W., Vigna, S. R., Clark, N. B., and Ralph, C. L. (1983). "Comparative Endocrinology." Wiley, New York.

- Heldmaier, G., Steiger, R., and Ruf, T. (1993). Suppression of metabolic rate in hibernation. In "Life in the Cold" (C. Carey, G. L. Florant, B. A. Wunder, and B. Horwitz, Eds.), pp. 545-548. Westview Press, Boulder, CO.
- Hellgren, E. C., Vaughan, M. R., Kirkpatrick, R. L., and Scanlon, P. F. (1990). Serial changes in metabolic correlates of hibernation in female black bears. *J. Mammal.* **71**, 291-300.
- Hellgren, E. C., Vaughan, M. R., Gwazdauskas, F. C., Williams, B., Scanlon, P. F., and Kirkpatrick, R. L. (1991). Endocrine and electrophoretic profiles during pregnancy and nonpregnancy in captive female black bears. *Can. J. Zool.* **69**, 892-898.
- Hissa, R., Siekkinen, J., Hohtola, E., Saarela, S., Hakala, A., and Pudas, J. (1994). Seasonal patterns in the physiology of the European brown bear (*Ursus arctos*) in Finland. *Comp. Biochem. Physiol. A* **109**, 781-791.
- Hulbert, A. J. (1978). The thyroid hormones: A thesis concerning their action. *J. Theor. Biol.* **73**, 81-100.
- Hulbert, A. J., and Hudson, J. W. (1976). Thyroid function in a hibernator, *Spermophilus tridecemlineatus*. *Am. J. Physiol.* **230**, 1211-1216.
- Irvine, C. H. G. (1967). Thyroid secretion rate in the horse in various physiological states. *J. Endocrinol.* **39**, 313-320.
- Lundberg, D. A., Nelson, R. A., Wahner, H. W., and Jones, J. D. (1976). Protein metabolism in the black bear before and during hibernation. *Mayo Clinic Proc.* **51**, 716-722.
- Magnus, T. H., and Henderson, N. E. (1988a). Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. I. Increased binding of triiodo-L-thyronine and L-thyroxine by serum proteins. *Gen. Comp. Endocrinol.* **69**, 352-360.
- Magnus, T. H., and Henderson, N. E. (1988b). Thyroid hormone resistance in hibernating ground squirrels, *Spermophilus richardsoni*. II. Reduction of hepatic nuclear receptors. *Gen. Comp. Endocrinol.* **69**, 361-371.
- Mead, R. A. (1989). The physiology and evolution of delayed implantation in carnivores. In "Carnivore Behavior, Ecology, and Evolution" (J. L. Gittleman, Ed.), pp. 437-464. Comstock/Cornell Univ. Press, Ithaca, NY.
- Nelson, R. A., Steiger, D. L., and Beck, T. D. I. (1983). Neuroendocrine and metabolic interactions in the hibernating black bear. *Acta Zool. Fenn.* **174**, 137-141.
- Nelson, R. A., Wahner, H. W., Jones, J. D., Ellefson, R. D., and Zollman, P. E. (1973). Metabolism of bears before, during, and after winter sleep. *Am. J. Physiol.* **224**, 491-496.
- Ramsay, M. A., and Dunbrack, R. L. (1986). Physiological constraints on life history phenomena: The example of small bear cubs at birth. *Am. Nat.* **127**, 735-743.
- Stolte, L., Kock, H., van Kessel, H., and Kock, L. (1966). Thyroxine utilization in non-pregnant, steroid-induced pseudopregnant, and pregnant monkeys. *Acta Endocrinol.* **52**, 383-390.
- Tomasi, T. E. (1991). Utilization rates of thyroid hormones in mammals. *Comp. Biochem. Physiol. A* **100**, 503-516.
- Tomasi, T. E., and Stribling, A. M. (1996). Thyroid function in the 13-lined ground squirrel. In "Adaptations to the Cold" (F. Geiser, A. J. Hulbert, and S. C. Nicol, Eds.), pp. 263-269. New England Press, Armidale, N.S.W.
- Wills, P. I., and Schindler, W. J. (1970). Radiothyroxine turnover studies in mice: Effects of temperature, diet, sex, and pregnancy. *Endocrinology* **86**, 1272-1280.
- Wimsatt, W. A. (1963). Delayed implantation in the Ursidae, with particular reference to the black bear (*Ursus americanus* Pallas). In "Delayed Implantation" (A. C. Enders, Ed.), pp. 49-76. Chicago Univ. Press, Chicago.