PLASMA LEPTIN AND BODY COMPOSITION IN POST-HIBERNATION THIRTEEN-LINED GROUND SQUIRRELS

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ABSTRACT

Leptin is a protein hormone primarily produced by white adipose tissue that is involved in numerous physiological processes, but is most often associated with regulation of energy intake and expenditure. Previous experiments have demonstrated that in some species, high circulating levels of leptin result in increased energy expenditure, decreased food intake, and a loss of lipid mass. However, little is known about the relationship between body composition and leptin in hibernators. We characterized plasma leptin levels and body composition of captive thirteen-lined ground squirrels *(Spermophilus tridecemlineatus)* 10, 15 and more than 20 days post-hibernation. Plasma leptin concentration was positively correlated with body mass, lipid mass, and lean mass. Percentage lipid significantly increased by 15 days post-hibernation and both plasma leptin values and lipid mass increased significantly by 20 days post-hibernation. These results indicate that within post-emergent thirteen-lined ground squirrels patterns of leptin secretion are similar to patterns described for other hibernating species.

Keywords

Hibernation, body composition, leptin, South Dakota, Spermophilus tridecemlineatus

Leptin is a protein hormone primarily produced by white adipose tissue and to a lesser extent by placental tissue, bone marrow, stomach, brain, and muscle tissue (Baile et al. 2000, Considine 2001). Leptin is synthesized by adipocytes in proportion to the amount of endogenous lipid (Baile et al. 2000) but plasma concentrations are also regulated by insulin, glucose, and sex hormones (Baile et al. 2000, Considine 2001). Leptin sensitivity is noted in some species to change with season (Boyer et al. 1997, Ormseth et al. 1997, Rousseau et al. 2003), and diet (Harris et al. 2003, Hope et al. 1999). Although implicated in the regulation a myriad of physiological process including thermogenesis, gonadotropin secretion, and immune function (Baile et al. 2000, Yu et al. 1995), its most prominent role is in the regulation of food intake and energy expenditure. Generally, elevated levels of plasma leptin result in decreased food consumption, reduced stores of body fat, and increased energy expenditure (Pelleymounter et al. 1995); therefore, it was theorized that the physiological role of leptin was to prevent obesity (Weigle et al. 1995). More recently however, Flier (1998) hypothesized that the physiological role of leptin is to signal the "switch" between levels of sufficient and insufficient energy stores rather than preventing significant changes in body composition.

To date, the majority of leptin studies have used rats or mice as model organisms. Because hibernators show high amplitude circannual changes in body mass, body composition, and energy metabolism (Buck and Barnes 1999a, Michener 1984, Michener and Locklear 1990) they are a unique model for studying the relationship between plasma leptin levels and body composition. Hibernators of the genus *Spermophilus* undergo two episodes of profound hyperphagia per annum (Buck and Barnes 1999a). Because they neither eat nor drink during hibernation, which in some species may last for more than 240 days, they must accumulate significant endogenous fuel stores during their prehibernation season to ensure their overwinter survival (Buck and Barnes 1999b, MacCarley 1966). Endogenous fuels are conserved during hibernation through profound reductions of body temperature and metabolic rate (Buck and Barnes 2000, Wang 1979). The reproductive season of ground squirrels immediately follows the termination of hibernation in early spring. This is the most energetically expensive portion of their annual cycle (Kenagy 1987, Kenagy et al. 1990); therefore, they must quickly recover lost energy stores in order to fuel the physiology and behavior associated with reproduction (Buck and Barnes 1999a).

One could argue that it would be maladaptive for hibernators to show a "typical" behavioral or physiological response to plasma leptin levels since this would preclude the circannual, rheostatic control of appetite and body condition. The only data examining plasma leptin concentration and body condition in obligate hibernators have been collected from two species, the arctic ground squirrel (*Spermophilus parryii*, Boyer et al. 1997, Ormseth et al. 1996) and the woodchuck (*Marmota monax*; Concannon et al. 2001). Moreover, these current data on the role of leptin in the regulation of body condition in ground dwelling sciurids are confounding and might change significantly with season. For example, Ormseth et al. (1996) demonstrated that a continuous infusion of mouse recombinant leptin over a 3-week period to captive arctic ground squirrels during their pre-hibernation fattening period significantly reduced both food intake and body weight as compared to control animals and baseline values. Yet, a similar infusion regime with arctic ground squirrels during their post-hibernation fattening period in spring did not decrease food intake or body mass (Boyer et al. 1997). Furthermore, peak plasma leptin concentration of arctic ground squirrels in spring was significantly higher than in fall (Boyer et al. 1997, Ormseth et al. 1996) and were much higher on average than in the woodchuck that exhibited no spring peak in plasma leptin concentration (Concannon et al. 2001).

This study is focused on thirteen-lined ground squirrels *(Spermophilus tridecemlineatus)* captured in southeast South Dakota. Thirteen-lined ground squirrels are small, grass-lands dwelling Spermophiles that exhibit, like other ground dwelling sciurids, significant circannual changes in appetite, body mass, condition, metabolism, body temperature, and activity (Hall 1981, Meyer and Morrison 1960, Michener 1984, Walker et al. 1994). The aim of our study was to characterize the relationship between plasma leptin levels and body composition of post-hibernation thirteen-lined ground squirrels.

METHODS

Animals. We live-trapped thirteen-lined ground squirrels *(Spermophilus tridecemlineatus)* in September and early October of 1999 and 2000 using Tomahawk® traps baited with carrot and peanut butter in Southeastern South Dakota, near the Lewis and Clark State Recreation Area (42° 92' N, 96° 39' W). Following capture, animals were transported to an animal holding facility at The University of South Dakota. Animals where individually housed in hanging metal cages in a 12:12 LD photoperiod and at an ambient temperature (T_a) of 25°C before the start of the experiment. Food (rodent chow, sunflower seed, and carrot) and water were provided *ad libitum* for the duration of the experiment. At the start of the hibernation season in late October 2000, we placed animals into either 15-L NALGENE® tubs or hanging metal cages and transferred them to an environmental chamber with a 0:24 LD photoperiod and T_a 0.8 ± 0.6°C (mean ± *SD*) . Following termination of hibernation in spring, we transferred animals from the environmental chamber to the animal holding facility and maintained in a 12:12 LD photoperiod and T_a 25°C and provided with food and water *ad libitum*. The University of South Dakota Institutional Animal Care and Use Committee approved all experiments and conditions of husbandry.

Blood samples and body composition. We collected blood and euthanized animals 10 (n = 4), 15 (n = 5), or greater than 20 (n = 5; range 20-25 days) days post-hibernation. We anesthetized animals with isoflurane vapors using bell-jar technique, weighed them to the nearest 0.5 g and collected between 2.5 and 3 ml of blood by cardiac puncture using non-heparinized 25 gauge needles and 3 cc syringes. Immediately following collection, blood was transferred to vials containing EDTA, centrifuged at approximately 3000 rpm for 10 minutes, and the plasma portion was drawn off and stored at –70 ° C until assayed. We determined body composition of animals by homogenizing whole frozen carcasses with a meat grinder, drying two portions of approximately 10 g each to constant mass in a drying oven (60°C, 24 h), and extracting lipids using a Soxtec® apparatus with 50-70 ml petroleum ether. We calculated total lipid and lean mass by extrapolating average percentages of lipid and lean of the two portions to the whole animal mass.

Plasma leptin concentration. Plasma leptin levels were measured by Linco Research, Inc (St. Charles, MO) using Linco Multi-Species radioimmunoassay kit (XL-85K) that has a minimal detectable level of 1.0 ng/ml. All samples analyzed fell within the detectable range of the assay for leptin

Statistical evaluation. We have presented all data as mean ± *SEM* unless otherwise stated and arcsine transformed percentage data prior to analysis. When assumptions of normality were not met, we square root transformed those data prior to analysis. We used a Student's *t*-test or one-tailed paired *t*-test for comparisons between two groups and one-way ANOVA followed by Tukey's test for pairwise comparisons of multiple groups of parametric data. We used the Kruskal-Wallis test for analysis of multiple groups with non-parametric data and a Pearson Product-Moment Correlation to test for significant correlation between variables. All differences were considered significant at $P < 0.05$.

RESULTS

Male and female thirteen-lined ground squirrels did not differ in plasma leptin concentration (t = -0.053, *d.f.* = 12, P = 0.959), body mass (t = 0.714, *d.f.* = 12, P = 0.489), lipid mass (t = -0.533, *d.f.* = 12, P = 0.603) percent lipid (t = -1.24, *d.f.* = 12, P = 0.240), or lean mass (t = 0.974, *d.f.* = 12, P = 0.349). Therefore, we pooled data from males and females for subsequent analyses. Groups of animals assigned to each of three sample period groups (10, 15, <20 days post-hibernation) did not significantly differ in body mass prior to the onset of hibernation and averaged 220.6 ± 15.57g ($F_{2,11}$ = 0.525, P = 0.606; Fig. 1). Body mass lost during hibernation was quickly recouped and returned to levels not significantly different from pre-hibernation levels by 15 d post-hibernation (Fig.1). Plasma leptin positively correlated with body mass (r = 0.766, P = 0.001; Fig. 2), grams of lipid (r = 0.844, P = 0.001; Fig. 2), percent lipid (r = 0.608, P = 0.02), and grams of lean (r = 0.627, P = 0.02; Fig. 2). Plasma leptin concentration ($F_{2,11}$= 6.925, P = 0.011) and lipid mass ($F_{2,11}$ = 4.313, P = 0.041) increased significantly by 20 days post-hibernation (Fig. 3). Percentage lipid significantly increased by 15 days post-hibernation (F = 5.629, *d.f.* = 2, 11, P = 0.029; q = 4.221, P< 0.05). Neither post-hibernation body mass

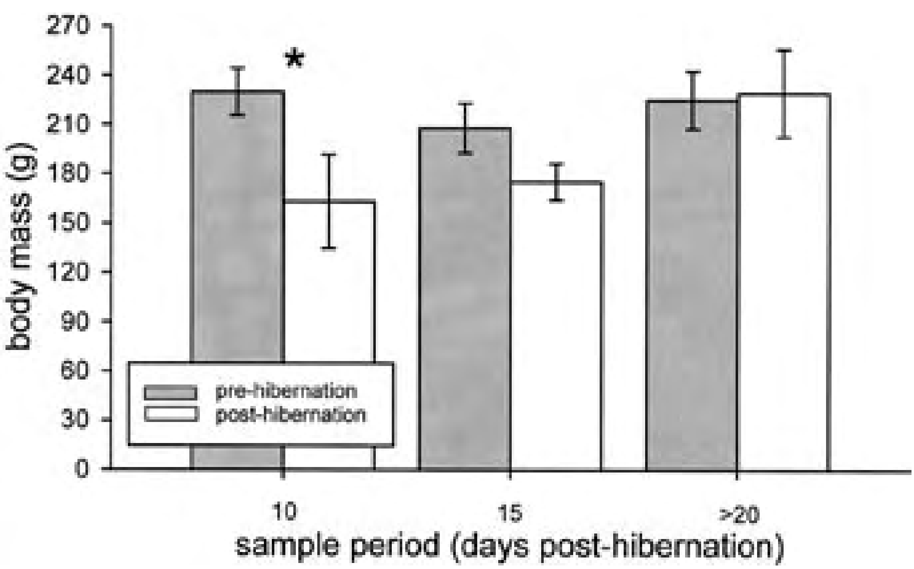

Figure 1. Body mass ± SE of thirteen-lined ground squirrels prior to onset of hibernation and 10, 15, and >20 days post-hibernation. Values within sample periods denoted by (*) are significantly different at P < 0.05. Points represent means ± SEM; n = 4 for 10 days post hibernation and n = 5 for 15 and >20 days post hibernation.

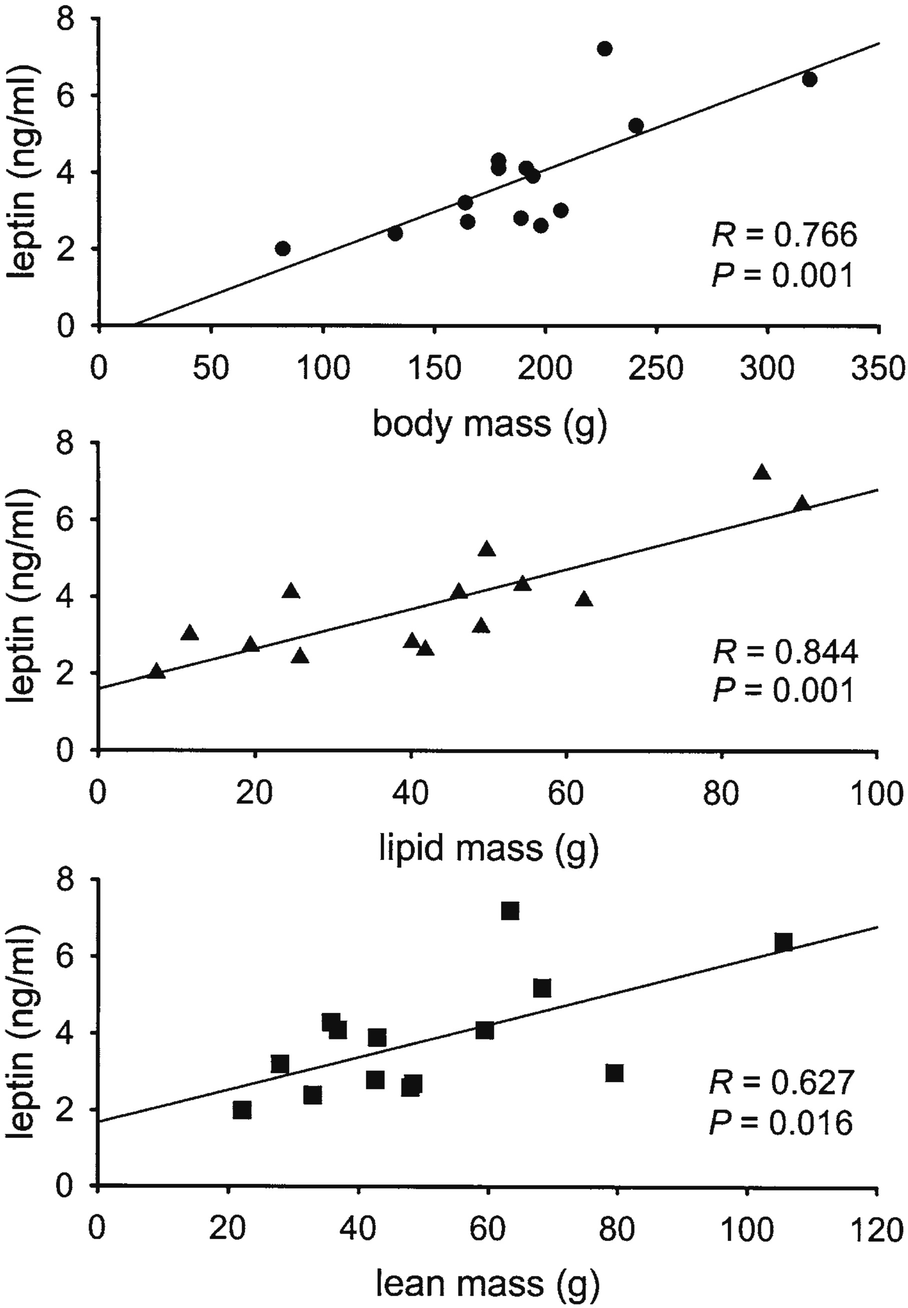

Figure 2. Plasma leptin concentration (ng/ml), body mass, lipid mass, and lean mass of individual thirteen-lined ground squirrels between 10 and >20 days of ending hibernation (n = 14).

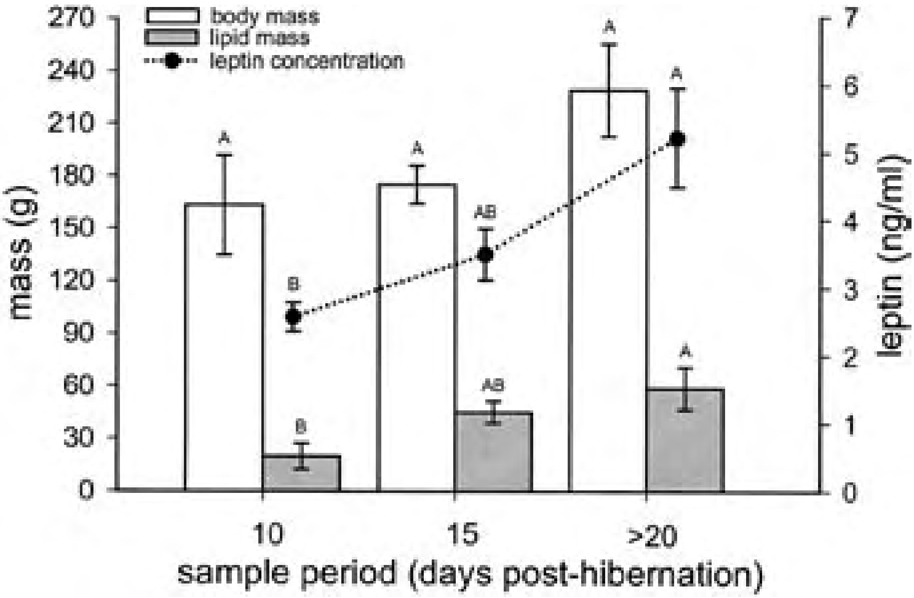

Figure 3. Plasma leptin concentration (ng/ml), body mass and lipid mass of thirteen-lined ground squirrels 10, 15, and more than 20 days post-hibernation. Values within a parameter that share a common superscript are not significantly different at P < 0.05. Points represent means ± SEM; n = 4 for 10 days post hibernation and n = 5 for 15 and >20 days post hibernation.

(F = 2.426, *d.f.* = 2, 11, P = 0.134) nor lean mass (H = 3.31, *d.f.* = 2, P = 0.20) significantly differed among sampling periods.

DISCUSSION

Our results describe the change in body condition of thirteen-lined ground squirrels in the ca. 20 days subsequent to ending hibernation and relate these changes to plasma concentrations of leptin. Body condition and plasma leptin concentration increased significantly throughout the study period (Fig. 3). The patterns and amplitude of change in body condition and leptin concentration observed in this study are consistent with those of post-emergent arctic ground squirrels (Boyer et al. 1997, Buck and Barnes, 1999b). Likewise, peak levels of leptin in spring of thirteen-lined ground squirrels in this study were comparable to peak spring levels of leptin reported by Boyer et al. (1997; thirteen-lined ground squirrels = 5.2 ± 0.7 ng/ml; arctic ground squirrels 4.48 ± 1.15 ng/ml). These levels, however, are >10 fold higher than peak values reported for woodchucks *(Marmota monax)* at any time in their annual cycle (Concannon et al. 2001). Moreover, the rapidity at which thirteen-lined ground squirrels increased plasma leptin concentration and recouped body mass in spring following the termination of hibernation was much greater than for woodchucks but similar to that described for Richardson's ground squirrel (Michener 1992) and arctic ground squirrels (Buck and Barnes, 1999b). Plasma leptin concentration of thirteen-lined ground squirrels had more then doubled between 10 and 20 days post hibernation and body masses were not significantly different from fall measures within 15 days of ending hibernation. In comparison, woodchucks undergo a gradual rise in leptin concentration beginning in early spring and peaking in early fall (Concannon et al. 2001).

Woodchucks differ from both arctic ground squirrels and thirteen-lined ground squirrels in that they undergo a protracted season of mild hyperphagia to a single peak in body mass in the fall (Concannon et al. 2001). Arctic ground squirrels and thirteen-lined ground squirrels undergo two seasons of profound hyperphagia per annum—one in the spring coincident with the end of heterothermy just prior to reproduction and another in the fall immediately before immergence into hibernation. It is likely that the greater mass of the woodchuck coupled with the metabolic savings of hibernation enables sufficient endogenous energy storage to preclude the need for a vernal period of hyperphagia. Yet, it is unclear if or how leptin might be involved in this behavioral difference.

We could not detect a significant difference in lean mass or whole body mass of animals among sample periods. We suspect that this is a statistical artifact of low sample size and the variability associated with using animals randomly captured from a wild population. We were, however, able to detect strong and positive relationships between body mass and leptin concentration and lipid mass and leptin concentration (Fig. 2). Together, these observations are consistent with other studies that have demonstrated a positive relationship between adipose tissue mass and plasma leptin concentration (Considine et al. 1996, Frederich et al. 1995, Lonngvist et al. 1995, Maffie et al. 1995).

We found no difference in body condition or circulating leptin concentration between male and female ground squirrels. Sex effects on leptin concentration have not been reported in other studies of ground dwelling sciurids; however, among humans, females have significantly greater leptin levels than males with equal body fat mass . These differences are most likely due to estrogenic stimulation and androgenic inhibition of leptin production (Baile et a. 2000, Considine 2001).

Although the current study ended before leptin concentrations were sufficient to elicit any anorectic effect and suppression of further body condition change, our results clearly demonstrate a strong relationship between body condition and leptin concentration in thirteen-lined ground squirrels in the 20 days following the end of hibernation. These results are comparable to other studies on ground squirrels, but differences in timing and magnitude of the change in body condition and leptin concentration, as compared to woodchucks, bring to light potential differences in patterns of leptin secretion that are consistent with life history characteristics.

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