Reduced leptin concentrations are permissive for display of torpor in Siberian hamsters

David A. Freeman,1 Daniel A. Lewis,1 Alexander S. Kauffman,2 Robert M. Blum,3 and John Dark4

Departments of 1Psychology and 2Integrative Biology, University of California, Berkeley, California 94720-1650; and 3Department of Biological Sciences, Lehigh University, Bethlehem, Pennsylvania 18015

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Freeman, David A., Daniel A. Lewis, Alexander S. Kauffman, Robert M. Blum, and John Dark. Reduced leptin concentrations are permissive for display of torpor in Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 287: R97–R103, 2004; 10.1152/ajpregu.00716.2003.—A photoperiod with a short photophase induces a winterlike phenotype in Siberian hamsters that includes a progressive decrease in food intake and body mass and reproductive organ regression, as well as reversible hypothermia in the form of short-duration torpor. Torpor substantially reduces energy utilization and is not initiated until body mass, fat stores, and serum leptin concentrations are at their nadir. Because photoperiod-dependent torpor is delayed until fat reserves are lowest, leptin concentrations may be a permissive factor for torpor onset. This conjecture was tested by implanting osmotic minipumps into Siberian hamsters manifesting spontaneous torpor; the animals received a constant release of leptin or vehicle for 14 days. Exogenous leptin treatment eliminated torpor in a significant proportion of treated hamsters, whereas treatment with the vehicle did not. Similarly, endogenous serum leptin concentrations were markedly reduced in all animals undergoing daily torpor. Although simply reducing leptin concentrations below a threshold value is not sufficient for torpor initiation, reduced leptin concentrations nevertheless appear necessary for its occurrence. It is proposed that drastically reduced leptin concentrations provide a “starvation signal” to an as yet unidentified central mechanism mediating torpor initiation.

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Address for reprint requests and other correspondence: J. Dark, Dept. of Psychology, Box 1650, Univ. of California, Berkeley, CA 94720-1650 (E-mail: johndark@socrates.berkeley.edu).

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sary, but not a sufficient signal to initiate torpor, then leptin values will not differ between hamsters never entering torpor and torpid hamsters or within hamsters during torpor and on a day without torpor. In addition, hamsters that never entered torpor were food deprived for 24 h in an attempt to induce torpor; serum leptin concentrations and body mass changes were measured.

**MATERIALS AND METHODS**

**Animals**

Male and female Siberian hamsters (2–10 mo) that had been reared in our laboratory colony in an LP [14 light (L):10 dark (D) photoperiod] at T_a = 22 ± 1°C were housed in individual polycarbonate cages with wood shavings bedding and with water and food (Purina Rodent Chow no. 5015) available ad libitum.

**Surgery**

In experiment 1, Siberian hamsters underwent a brief surgical procedure to place radiotransmitters intra-abdominally for telemetric recording of T_b. Under deep anesthesia [0.34 ml ketamine cocktail/100 g body mass (cocktail = 21.0 mg ketamine + 2.4 mg xylazine + 0.3 mg acepromazine/ml)], the midline abdomen was shaved and a small midline incision was made between the scapulae, the minipump was inserted under the interscapular area during a brief surgery. A small midline incision was made over the leptin (details below) were subcutaneously implanted in the abdomen. The osmotic minipump of one hamster malfunctioned and this animal was discarded from the study.

**Leptin Treatment and Leptin Assay**

In experiment 1, hamsters anesthetized with isoflurane inhalant received osmotic minipumps [Alzet, model 2002, which provided 14 days of relatively constant release (0.5 μg/h) before reservoir exhaustion] containing leptin (Sigma; ~1 mg·kg⁻¹·day⁻¹ dissolved in PBS at pH 5.2) or PBS.

In experiment 2, hamsters were anesthetized with isoflurane cocktail, and radiotransmitters were implanted (as described above) for telemetrically recording T_b. After recovering from surgery, the animals were moved to an environmental chamber with a low T_a.

**Telemetric Recording of T_b and Criterion for Torpor**

Body temperature was recorded telemetrically from Minimitter transmitters (model VM-FH or VM-FH-LT, Minimitter, Sunriver, OR) implanted within the abdomen. Transmitter signals were captured by receiver boards under each animal’s cage and stored and translated every 10 min by computer program (Dataquest, St. Paul, MN). Criterion for torpor was a T_b ≤ 31°C for at least 30 min (6). Torpor duration was defined as the total time spent at T_b < 31°C; depth of torpor was gauged by the absolute minimum T_b (T_bmin) achieved during a torpor bout.

**Procedures**

**Experiment 1.** Body mass measures of 60 adult male (n = 30) and female (n = 30) Siberian hamsters housed in LP were obtained, and the animals were then moved into an SP (10L:14D photoperiod) and a T_a of ~22°C. After 12 wk of SP treatment, body mass was again recorded and pelage color was observed. Forty hamsters with reduced body mass and a clear transition to a gray-white winter pelage, an indication of photoresponsiveness (10), underwent a brief surgical procedure during which intra-abdominal radiotransmitters were implanted. On recovery from surgery, the animals were transferred into an environmental chamber with an identical SP and a T_a reduced to 15°C. Hamsters with established reliable records of torpor (minimum of 3 bouts) during a 14-day pretreatment period (n = 20) were removed from the cold for a brief surgical procedure (see above) in which an osmotic minipump was implanted interscapularly. Hamsters received minipumps containing leptin (n = 12) or PBS vehicle (n = 8). T_b records were examined for evidence of torpor during the 14 days of treatment and for the subsequent 14-day posttreatment period. The depth and duration of torpor bouts were determined.

**Experiment 2.** Forty-four adult female Siberian hamsters that had been maintained in an LP at 22°C were lightly anesthetized and weighed, and a blood sample was taken to establish baseline leptin concentration measurements. After 9 wk in SPs, hamsters were reweighed and a second blood sample was obtained. The hamsters had a radiotransmitter implanted for recording T_b, as described above. After surgery, the animals were moved to an environmental chamber with an identical SP but T_a = 15°C. The animals were then screened daily for the occurrence of torpor. Blood was sampled during torpor (T_b < 31°C) in a total of 10 hamsters. Serum was collected during the entry, or cooling, phase of torpor (i.e., as soon as T_b declined to <31°C) in eight of these animals and at approximately the same time in a day in which torpor was not displayed; for one-half of the animals the first blood sample was drawn on a day without torpor and for the rest on a day with torpor. A third sample was collected from some of these animals (n = 5) as they approached T_bmin (i.e., T_b = ~20–25°C). Blood samples also were collected at approximately the same time of day from animals that never entered torpor (n = 14). Subsequently, 10 animals that never displayed torpor were food deprived for 24 h beginning just before the onset of the dark phase. A serum sample for leptin analysis was obtained from these animals during torpor or, where no torpor was displayed, during the light phase toward the end of the normal time for torpor.

**Statistical Analysis**

Comparisons between and within groups were made using χ-square, Mann-Whitney ranked sum test, Wilcoxon signed ranks test, paired and unpaired t-tests, one-way ANOVA, or two-way ANOVA (with Student-Newman-Keuls paired comparisons when appropriate). Comparisons were considered statistically significant when P < 0.05.

**RESULTS**

**Experiment 1**

The osmotic minipump of one hamster malfunctioned and was still completely full of leptin at autopsy ~6–7 wk after minipump implantation; this animal was discarded from the study. Body mass at minipump implantation was not statistically different (t-test = 1.43, P > 0.05; not shown) between the Siberian hamsters treated with leptin ([27.0 ± 0.87, n = 11 (6 male and 5 female)] and those receiving PBS vehicle [25.4 ± 0.67, n = 8 (3 male and 5 female)]. The proportion of animals that entered torpor during the 14 days before treatment, 14 days during treatment, and 14 days after treatment with leptin was...
statistically different ($\chi^2 = 13.93, P < 0.05$), whereas there was no corresponding difference for those treated with PBS ($\chi^2 = 2.40, P > 0.05$; Fig. 1A). Despite a decreased proportion entering torpor, there was no statistical effect of leptin treatment (2-way ANOVA, $F_{1,19} = 3.51, P > 0.05$) or time (2-way ANOVA, $F_{1,19} = 0.16, P > 0.05$) on the depth of torpor (Fig. 1B). No statistical effect of leptin treatment (2-way ANOVA, $F_{1,19} = 8.15, P < 0.05$) or time (2-way ANOVA, $F_{1,19} = 0.29, P > 0.05$) was observed on the duration of torpor. Frequency did not differ between the leptin and PBS groups before (5.75 ± 1.03 vs. 4.83 ± 0.83, respectively; Student-Newman-Keuls, $P > 0.05$) or during treatment (2.50 ± 1.19 vs. 2.33 ± 0.96; Student-Newman-Keuls, $P > 0.05$).

**Experiment 2**

After 9 wk of SPs, body mass of Siberian hamsters ($n = 44$) decreased statistically from baseline LP values (Wilcoxon signed rank test, $P < 0.05$; Fig. 2A), and serum leptin concentrations were reduced by 74.5% (Wilcoxon signed rank test, $P < 0.05$; Fig. 2B). Body mass and serum leptin were positively correlated in LP (linear regression analysis: $R = 0.88$; $F_{1,42} = 139.93, P < 0.05$).
134.57, \( P < 0.05 \) and in SP hamsters (\( R = 0.44; F_{1,42} = 9.94, P < 0.05 \)).

Another body mass and leptin sample was obtained from 25 SP hamsters after nine additional weeks of SPs and \( T_a = 15^\circ C \). There was no additional effect on body mass (Wilcoxon signed rank test, \( P > 0.05 \); see Fig. 3A). Although euthermic serum leptin concentrations were further reduced by 25.3\%, this difference did not reach statistical significance, most likely due to high data variability (Wilcoxon signed rank test, \( P > 0.05 \); Fig. 3B).

The body mass of the 10 Siberian hamsters measured during torpor was statistically lower (\( t\)-test = 3.67, \( P < 0.05 \); Fig. 4A) than that of hamsters that never entered torpor (\( n = 14 \)). Although mean value of hamsters never torpid (range = 1.82–7.96 ng/ml) was 38.4\% greater than that of torpid hamsters (range = 1.61–2.57 ng/ml), serum leptin concentrations of the torpid hamsters were not statistically different from those of hamsters never torpid (Mann-Whitney rank sum test, \( P > 0.05 \); Fig. 4B). Linear regression analyses revealed a significant relation between leptin and body mass in torpid hamsters (\( R = 0.65; F = 5.97, P < 0.05 \)) but not in hamsters never entering torpor (\( R = 0.34; F = 1.58, P > 0.05 \)).

![Fig. 3. Body mass (mean ± SE; A) and serum leptin concentration (mean ± SE; B) of euthermic Siberian hamsters (\( n = 25 \)) in SP and ambient temperature (\( T_a = 22^\circ C \) (SP-Warm) and after 9 additional weeks in SP at \( T_a = 15^\circ C \) (SP-Cold).](image-url)

![Fig. 4. Body mass (mean ± SE; A) and serum leptin concentration (mean ± SE; B) of euthermic Siberian hamsters that never entered torpor (Never-Torpid, \( n = 14 \)) and hypothermic torpid hamsters (Torpid, \( n = 10 \)). *Statistically different from Never-Torpid.](image-url)

![Fig. 5. Mean (±SE) leptin concentration of the same hamsters (\( n = 8 \)) during torpor (Torpid) and at the same time of day on a day without torpor (Euthermic).](image-url)
leptin at torpor nadir (Tb = 20–25°C) also did not differ from values at torpor entrance or euthermia (repeated-measures ANOVA, F2,14 = 0.51, P > 0.05; Fig. 6).

Twenty-four hour food deprivation of 10 hamsters that never initiated torpor did not affect (Wilcoxon signed rank test, P > 0.05, not shown) body mass (before 30.14 ± 1.04 g, after 29.58 ± 1.54 g) nor did it alter (paired t-test = 1.54, P > 0.05; not shown) serum leptin concentrations (before 3.06 ± 0.61 ng/ml vs. after 2.29 ± 0.21 ng/ml). After food deprivation for 24 h, only one of the hamsters that previously had not entered torpor displayed the behavior (leptin = 2.43 ng/ml). Although the three lowest leptin values recorded (1.61, 1.59, and 1.48 ng/ml) were from three food-deprived hamsters, these hamsters did not initiate torpor.

**DISCUSSION**

Exogenous leptin but not vehicle treatment decreased the proportion of Siberian hamsters that entered torpor. We are not sure why some hamsters treated with leptin continued to exhibit torpor. One possibility is that the osmotic minipumps in some cases may not have provided sufficient increases in leptin to inhibit torpor; similar osmotic minipumps in a comparably sized laboratory mouse increased serum leptin concentrations over a broad range of values (3–14 ng/ml; Ref. 12). Surprisingly, the depth and duration of torpor in leptin-treated hamsters that underwent torpor was unaffected. Frequency of torpor in animals continuing to enter torpor also did not differ between leptin- and vehicle-treated hamsters. In addition, endogenous serum leptin concentrations and body mass were reduced in SP hamsters. Although our LP leptin values were somewhat higher, leptin concentrations after 9 and 18 wk of SP treatment were similar to those previously reported for the species at comparable time points (25). Endogenous leptin concentrations of Siberian hamsters entering torpor were very low. Leptin values of torpid hamsters in SPs and Ta enter 2°C (15°C) were reduced by 88% compared with euthermic hamsters in LPs at Ta = 22°C (19.03 ng/ml). No hamster entering torpor had a leptin concentration >2.57 ng/ml. A comparable scenario characterizes the hibernating little brown bat; endogenous leptin concentrations are decreased despite increased fat stores during the hibernation season, presumably to remove leptin’s inhibitory effects on hibernation (28).

Several lines of evidence suggest that reduced leptin concentrations did not directly trigger torpor, despite being a permissive factor for its occurrence. First, concentrations of leptin in torpid hamsters and hamsters never torpid in the cold were not statistically different; there was considerable overlap in the leptin values of torpid and persistently euthermic hamsters. Second, leptin concentrations were similar within the same hamsters during torpor and on a day without torpor. Third, leptin concentrations did not change in hamsters during the transition from euthermia (37°C) to torpor entrance (−31°C) to Tbmin (−20–24°C). Finally, although food deprivation of hamsters that never entered torpor elicited the three lowest leptin values in this study, these animals still did not initiate torpor. We suggest that chronically reduced leptin concentrations are a permissive factor for torpor occurrence. A reduced concentration of leptin below a certain threshold may be necessary for torpor to occur; nevertheless it is not a sufficient cue to trigger onset of a torpor bout. This is best evidenced by the complete lack of differences in serum leptin concentrations within the same animals during torpor entrance and on a day without torpor.

The effect of exogenous leptin on torpor has been previously evaluated in a marsupial (13). Leptin injections affected the expression of food restriction-induced torpor in the marsupial *Sminthopsis macroura* but with a difference. In this marsupial, leptin’s primary effect was to decrease duration of torpor and increase Tbmin by increasing metabolic rate (MR) (13). Presumably, the neuroendocrine mechanisms underlying torpor differ between placental and marsupial mammals because of their reliance on different mechanisms for thermoregulatory thermogenesis. BAT nonshivering thermogenesis (NST) is important for maintaining euthermia of Siberian hamsters in low temperatures (16, 18, 34); marsupials apparently do not possess thermoregulatory BAT (33) and must as a consequence rely on other mechanisms for thermoregulatory heat production. Nevertheless, exogenous leptin (and, presumably, high endogenous leptin) inhibits torpor expression in both a marsupial and, as confirmed here, in a placental mammal. Marsupial and placental mammals, which have been evolutionarily separated for over 100 million years, may have convergently evolved comparable tactics when facing a similar energetic challenge but with different underlying mechanisms.

Endotherms undergo a circadian cycle in Tb; in Siberian hamsters, as in other nocturnal rodents, Tb maxima occur during the dark, active phase and Tb minima occur during the light, sleep phase (e.g., 40, 42). Food restriction in mice enhances the normal circadian decline in Tb, reducing Tbmin even further and decreasing the underlying MR (8). Exogenous leptin treatment prevents this enhanced circadian Tbmin and increases MR in adult mice (8). A comparable elimination of a torporlike enhanced circadian Tbmin occurs in suckling rat pups after exogenous leptin treatment (5, 40). Similarly, leptin-deficient ob/ob mice evidence a much greater reduction in circadian Tb minima than their lean littermates during food restriction (19). Although exogenous leptin reversed the marked decrease in Tb of ob/ob mice during complete food deprivation, Tbmin occurred during the dark phase when mice are normally active (12). Exogenous leptin did not prevent extreme hypothermia in food-deprived A-ZIP/F-1 transgenic

![Fig. 6. Mean (±SE) leptin concentrations within Siberian hamsters (n = 5) during euthermia (Euthermic), entrance to torpor (Entering Torpor), and near minimum body temperature (Tb) (Torpor Nadir).](image-url)
mice at room temperature; however, these mice exhibit numerous serious physiological disturbances. They have little or no white adipose tissue, minimal BAT, low leptin concentrations, extremely high corticosterone concentrations, and higher than normal blood glucose concentrations despite being hyperinsulinemic (12). Their hypothermia may be torpor or it may possibly be an inability to maintain euthermic Tb.

Shallow, daily torpor is an exaggeration of the normal rest, sleep phase decrease in circadian Tb (7, 15, 52). Spontaneous and food restriction-induced daily torpor are believed to have developed from the normal sleep phase reductions in circadian Tb_{min}. High leptin values for the most part suppressed torpor in Siberian hamsters (present study). Mice and preweaning rat pups can undergo reversible hypothermia in the form of an exaggerated circadian Tb_{min} when energetically challenged; this effect also is blocked by exogenous leptin (5, 8, 48). High leptin concentrations appear to generally counter hypothermic energy-saving reductions in Tb and MR.

Although leptin is believed to increase MR in laboratory rats and mice by facilitating sleep phase thermogenesis, specifically BAT NST (1, 35, 50), this would not appear to be the case in those Siberian hamsters continuing to undergo spontaneous torpor. Exogenous leptin eliminated torpor in the majority of animals, but neither the depth nor the duration of torpor was affected in the few leptin-treated animals still entering torpor (experiment 1). If leptin were directly affecting thermogenesis, then there should have been an increase in Tb_{min} and a decrease in torpor duration as observed by Geiser et al. (13) in torpid marsupials. This conclusion is also supported by the lack of a relation between leptin concentration and the occurrence and/or depth of torpor (experiment 2). Similarly, elevated leptin values prevented enhanced decreases in Tb_{min} in rat pups and mice but did not increase Tb_{max} (8, 48). It appears that in all the above cases leptin prevents an adaptive selective decrease in circadian Tb_{min} set point; leptin would not appear to simply increase thermogenic output.

Exposure to SPs results in reduced food intake and body mass and inhibition of numerous reproductive indexes within several weeks, reaching trough values after 12–15 wk (4, 11, 30). Photoperiod-dependent torpor, however, is not initiated until at least ~12 wk of SPs (e.g., Ref. 14), when body mass (4), white adipose tissue reserves (3), and serum leptin concentrations are lowest (2, 25, present study). It has been speculated that leptin feedback to the brain provides a “starvation signal,” reflecting the depletion of energy reserves (e.g., 1, 12). This starvation signal appears to be a precursor for spontaneous torpor in SPs. Food restriction of Siberian hamsters in LPs reduces body mass and serum leptin concentrations (39) and can also induce torpor (41, 42), which apparently does not occur until body mass has decreased below a critical level (39, 41). It is unknown at this time whether reduced leptin values are also a necessary precondition for food restriction-induced torpor in LPs.

Exogenous leptin may be less effective in reducing food intake, body mass, and fat depot mass of Siberian hamsters in long than short photoperiods (25, 36, 37). The photoperiodic mechanism underlying other seasonal changes in behavior and physiology may also mediate altered sensitivity to leptin feedback (38). To date, however, the effect of exogenous leptin in different photoperiods has only been tested for food intake and/or body mass and fat. Neural sensitivity to leptin feedback may be unchanged with regard to its effect on circadian Tb. Leptin primarily provides feedback to the brain via the arcuate nucleus (ARC), inhibiting NPY (a potent orexigenic factor) activity (e.g., 50). One of the consistent characteristics of Siberian hamsters in SPs is that concentrations of NPY mRNA do not decrease despite the presence of hypophagia (31, 32, 36, 37). Additionally, ARC agouti-related peptide and cocaine-and amphetamine-related transcript also do not change in SP. Proopiomelanocortin (POMC) in the ARC surprisingly decreases in SP. Because it acts indirectly to inhibit food intake and increase energy expenditure (via α-melanocyte-stimulating hormone), this change should be counteractive to hypophagia. This suggests that SP-dependent changes and food intake are independent of ARC peptides and probably mediated by other neural mechanisms (see Refs. 11, 32). Unchanged NPY and decreased POMC activity in the ARC, on the other hand, may augment decreases in circadian Tb characteristic of torpor. NPY, which can act on thermogenesis independently of its effect on food intake (26, 27), inhibits BAT NST, reducing Tb and MR (22, 51).

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