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

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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.

thermoregulation; hypothermia; energy balance; adipose tissue; photoperiod

ENERGY BALANCE is accomplished through a delicate equating of energy intake (food consumption) and energy expenditure (calorie-fueled cellular oxidation). In mammals, a majority of the energy budget is committed to maintaining homeothermia [i.e., body temperature (T_b) = ~37°C]. This is particularly true for small mammals living in temperate or boreal or austral environments, including the Siberian hamster (*Phodopus sungorus*). During the cold winter months, the increased demand for energy intake due to higher thermogenic costs occurs when food availability and/or quality also are likely to be lowest (see Ref. 53). To contend with this circumstance, some endothermic mammals periodically abandon homeothermia in favor of heterothermia, spending several hours with a T_b as low as 15°C (shallow, daily torpor) or up to several weeks with a T_b as low as 0°C (hibernation) (29). Heterothermia in the Siberian hamster is of the former type (17) and provides a considerable energetic savings, reducing daily energy utilization by as much as 24% with a single bout of torpor (43). The reduction in energy expenditure, in turn, reduces energy intake requirements (6, 23, 44) and, presumably, the time spent above ground foraging, a major energy drain during winter. The savings accrued from torpor may be the difference between overwinter survival and death in small mammals.

The peptide leptin (which originates primarily in white adipose tissue) is an important signal that provides feedback to neural mechanisms controlling adiposity, food intake, and energy expenditure (for reviews, see Refs. 1, 35, 50). Exogenous leptin treatments decrease adiposity by decreasing food intake and by increasing energy utilization (8, 21). High leptin concentrations increase sleep-phase thermoregulatory thermogenesis, specifically brown adipose tissue (BAT) capacity and, possibly, activity (46, 49). Torpor involves a temporary suspension of thermogenesis; therefore high circulating concentrations of leptin may prevent regulated hypothermia.

When Siberian hamsters are maintained in winterlike photoperiods with a short photophase (SP), they undergo spontaneous decreases in food intake and body mass (20) and a marked depletion of fat stores (3). The latter presumably underlies the decreases in white and brown adipose tissue leptin mRNA (24, 32) and circulating leptin concentrations seen in SPs (2, 9). Siberian hamsters do not begin to express spontaneous daily torpor until the body mass trough is nearly achieved (14, 45). Similarly, food restriction-induced torpor in a long photophase (LP) must produce a comparable decrease in body mass before torpor will occur (39, 42). Reduced leptin concentrations subsequent to photoperiod- or food restriction-induced fat mass loss may be a necessary prerequisite and/or sufficient signal for the occurrence of torpor.

If reduced circulating leptin concentrations are a necessary feedback signal on which the expression of torpor is dependent, then Siberian hamsters undergoing spontaneous SP-induced torpor should suspend torpor during treatment with leptin. To test this conjecture, adult Siberian hamsters housed in an SP and low ambient temperature (T_a) and exhibiting spontaneous torpor were administered exogenous leptin for ~14 days via osmotic minipumps and were monitored for the presence or absence of torpor. If exogenous leptin treatment inhibits or blocks torpor, as is expected, then determining endogenous leptin concentrations before and during spontaneous torpor becomes of considerable importance. Not all Siberian hamsters undergo torpor after prolonged exposure to SPs; as a result, serum leptin concentrations can be compared in animals that do and do not enter torpor. Leptin concentrations can also be measured in individual hamsters on days with and without torpor. If reduced leptin concentrations are a neces-

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 $T_a = 22 \pm 1$°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 short incision was made. The transmitter was inserted into the peritoneum, and the peritoneum and skin were then closed with sterile sutures. The wound was treated with 0.1% nitrofurazone ointment. The animals were treated with bupromorphine (0.05 mg/kg body mass coinjected at the time of anesthetization) to alleviate postsurgical discomfort. Several weeks later (see *Procedures*), hamsters were anesthetized with isoflurane inhalant, and osmotic minipumps containing leptin (details below) were subcutaneously implanted in the interscapular area during a brief surgery. A small midline incision was made between the scapulae, the minipump was inserted under the skin, and the wound was closed with several sterile sutures.

In *experiment 2*, hamsters were anesthetized with ketamine 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.

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 μl/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 vapors, and blood samples (~250 μl) were collected from the retroorbital sinus using a capillary tube. Blood samples were kept on ice until frozen at −80°C and later assayed for leptin. Leptin concentrations were determined using the Linco multispecies leptin assay, previously used to measure leptin in Siberian and Syrian hamsters (9, 47).

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 $T_b \le 31$°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 ($T_{b|min}$) 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 on 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 $T_{b|min}$ (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. 1*A*). 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
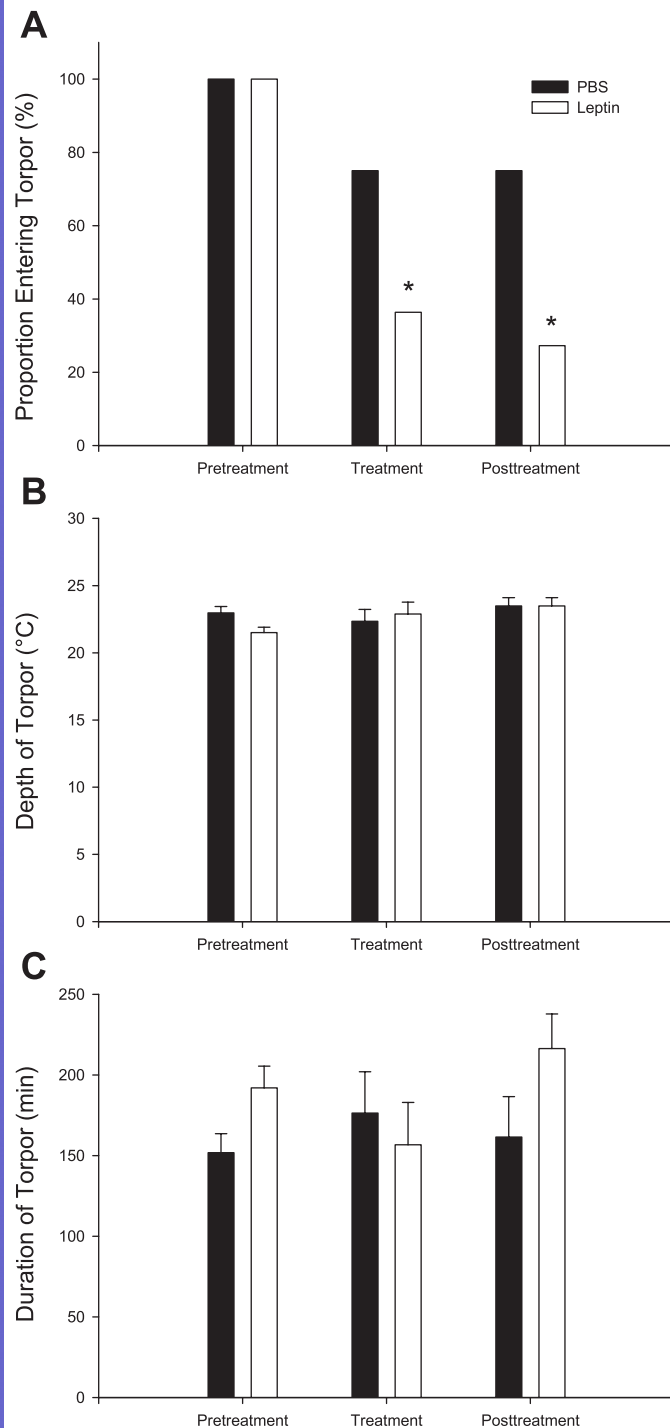

Fig. 1. *A*: proportion of Siberian hamsters entering torpor for the 14 days before treatment, 14 days during treatment, and 14 days after treatment with leptin ($n = 11$) or PBS vehicle ($n = 8$). *Statistically significant change in proportion of animals entering torpor. *B*: depth of torpor (mean ± SE) before, during, and after leptin or PBS treatment. *C*: duration of torpor (mean ± SE) during pretreatment, treatment, and posttreatment.
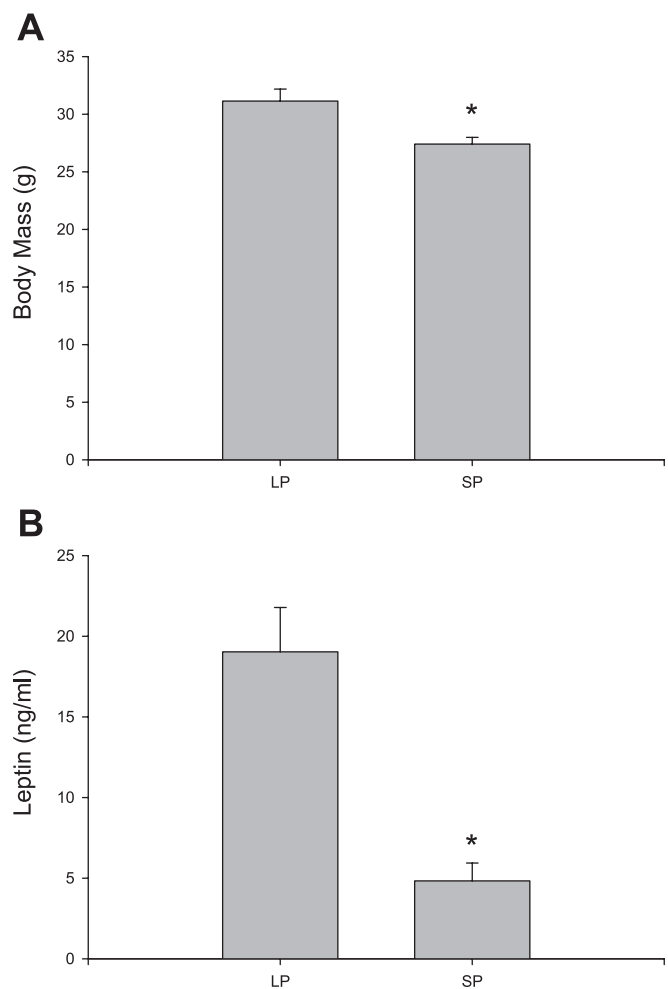

Fig. 2. Body mass (mean ± SE; *A*) and serum leptin concentration (mean ± SE; *B*) of Siberian hamsters ($n = 44$) measured in long photophase (LP) and then after 9 wk in short photophase (SP). *Statistically different from LP value.

ANOVA, $F_{1,19} = 0.16$, $P > 0.05$) on the depth of torpor (Fig. 1*B*), nor was the duration of torpor affected by either treatment (2-way ANOVA, $F = 1.78$, $P > 0.05$; Fig. 1*C*) or time (2-way ANOVA, $F = 0.44$, $P > 0.05$). In those hamsters continuing to enter torpor during or after treatment, time (before vs. after minipump implantation) reduced torpor frequency (bouts/14 days) in both groups (2-way ANOVA, $F_{1,19} = 8.15$; $P < 0.05$, not shown), but treatment (leptin vs. PBS) failed to affect torpor frequency (2-way ANOVA, $F_{1,19} = 0.29$; $P > 0.05$, not shown). 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. 2*A*), and serum leptin concentrations were reduced by 74.5% (Wilcoxon signed rank test, $P < 0.05$; Fig. 2*B*). Body mass and serum leptin were positively correlated in LP (linear regression analysis: $R = 0.88$; $F_{1,42} =$

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 $T_a = 15°C$. 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 ($R = 0.34$; $F = 1.58$, $P > 0.05$).
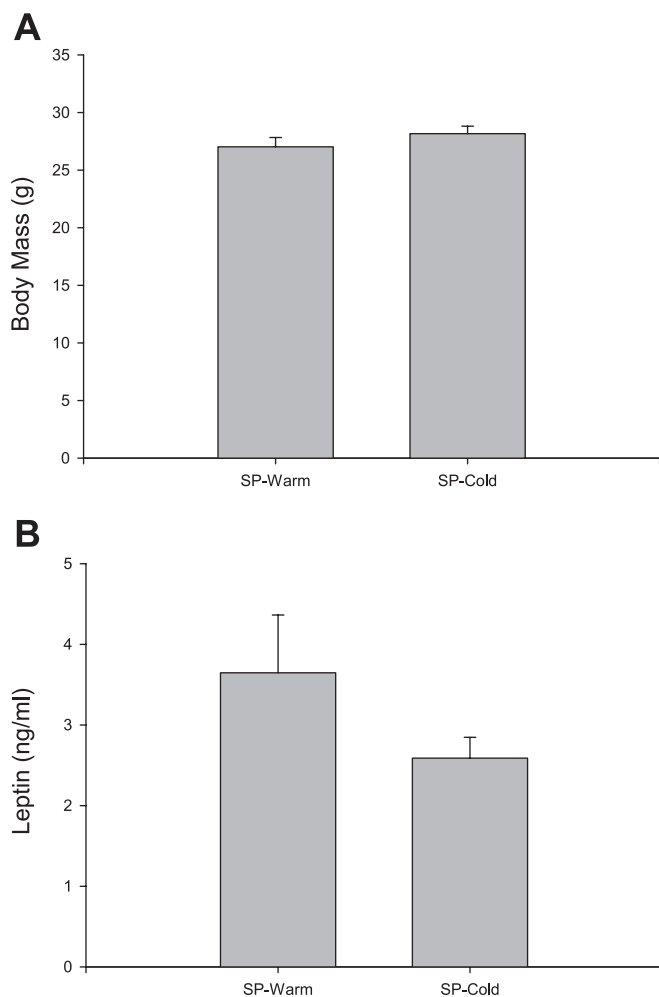

Fig. 3. Body mass (mean $\pm$ SE; A) and serum leptin concentration (mean $\pm$ SE; B) of euthermic Siberian hamsters ($n = 25$) in SP and ambient temperature (T_a) $= 22°C$ (SP-Warm) and after 9 additional weeks in SP at $T_a = 15°C$ (SP-Cold).
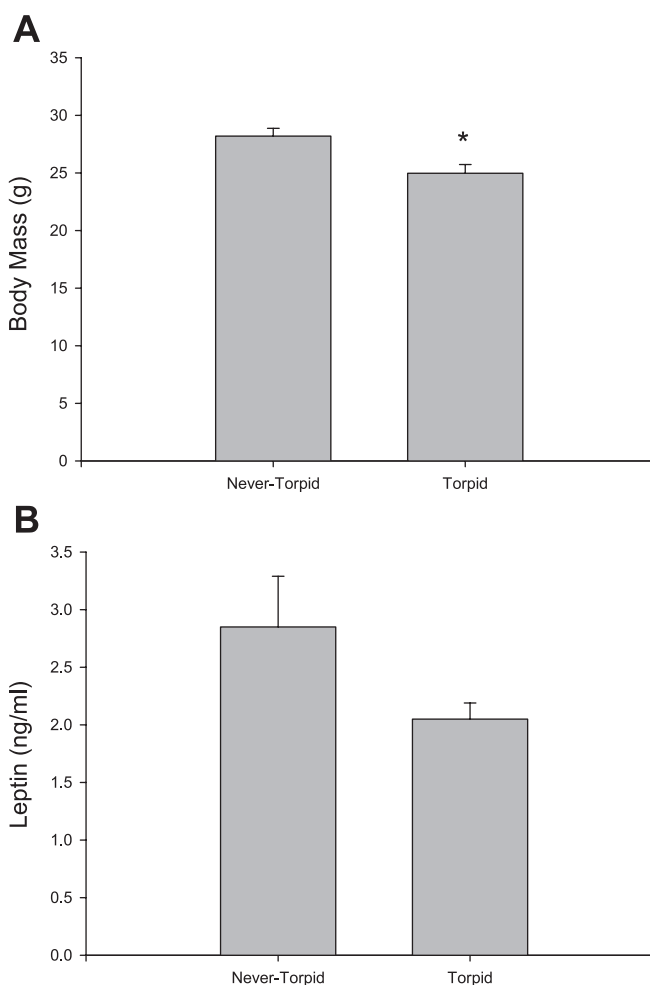

Fig. 4. Body mass (mean $\pm$ SE; A) and serum leptin concentration (mean $\pm$ SE; B) of euthermic Siberian hamsters that never entered torpor (Never-Torpid, $n = 14$) and hypothermic torpid hamsters (Torpid, $n = 10$). *Statistically different from Never-Torpid.

Serum leptin concentrations in eight hamsters was comparable during torpor entrance ($T_b < 31°C$) and at the same time of day on a day without torpor ($T_b = \sim37°C$) (paired t-test $= 1.21$, $P > 0.05$; Fig. 5). Additionally, in five of these hamsters
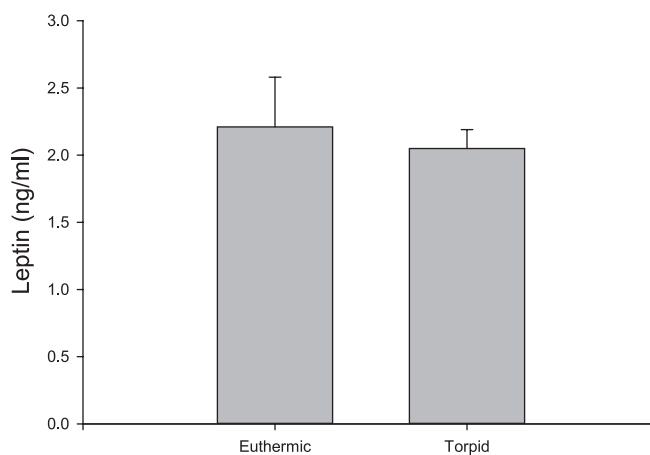

Fig. 5. Mean ($\pm$SE) leptin concentration of the same hamsters ($n = 8$) during torpor (Torpid) and at the same time of day on a day without torpor (Euthermic).

leptin at torpor nadir (T_b = 20–25°C) also did not differ from values at torpor entrance or euthermia (repeated-measures ANOVA, $F_{2,14}$ = 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 vs. 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 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 (lepti$n$ = 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 T_a = 15°C (2.06 ng/ml) were reduced by 88% compared with euthermic hamsters in LPs at T_a = 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 $T_{b|min}$ (~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 $T_{b|min}$ 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 T_b; in Siberian hamsters, as in other nocturnal rodents, T_b maxima occur during the dark, active phase and T_b minima occur during the light, sleep phase (e.g., 40, 42). Food restriction in mice enhances the normal circadian decline in T_b, reducing $T_{b|min}$ even further and decreasing the underlying MR (8). Exogenous leptin treatment prevents this enhanced circadian $T_{b|min}$ and increases MR in adult mice (8). A comparable elimination of a torporlike enhanced circadian $T_{b|min}$ occurs in suckling rat pups after exogenous leptin treatment (5, 40). Similarly, leptin-deficient *ob/ob* mice evidence a much greater reduction in circadian T_b mimima than their lean littermates during food restriction (19). Although exogenous leptin reversed the marked decrease in T_b of *ob/ob* mice during complete food deprivation, $T_{b|min}$ 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
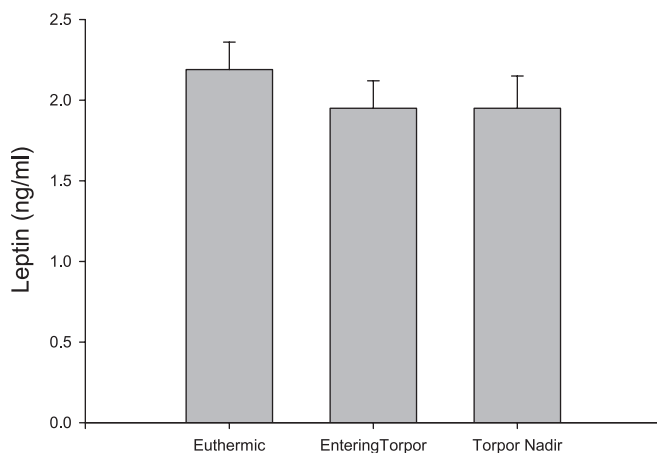

Fig. 6. Mean (±SE) leptin concentrations within Siberian hamsters (n = 5) during euthermia (Euthermic), entrance to torpor (Entering Torpor), and near minimum body temperature (T_b) (Torpor Nadir).

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 T_b.

Shallow, daily torpor is an exaggeration of the normal rest, sleep phase decrease in circadian T_b (7, 15, 52). Spontaneous and food restriction-induced daily torpor are believed to have developed from the normal sleep phase reductions in circadian $T_{b|min}$. 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 $T_{b|min}$ 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 T_b 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 $T_{b|min}$ 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 T_b mimima in rat pups and mice but did not increase T_b maxima (8, 48). It appears that in all the above cases leptin prevents an adaptive selective decrease in circadian $T_{b|min}$ 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 T_b. 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 T_b characteristic of torpor. NPY, which can act on thermogenesis independently of its effect on food intake (26, 27), inhibits BAT NST, reducing T_b and MR (22, 51).

REFERENCES

1. **Ahima RS and Flier JS.** Leptin. *Annu Rev Physiol* 62: 413–437, 2000.
2. **Atcha Z, Cagampang FR, Stirland JA, Morris ID, Brooks AD, Ebling FJ, Klingenspor M, and Loudon AS.** Leptin acts on metabolism in a photoperiod-dependent manner, but has no effect on reproductive function in the seasonally breeding hamster (*Phodopus sungorus*). *Endocrinology* 141: 4128–4135, 2000.
3. **Bartness TJ, Hamilton JM, Wade GN, and Goldman BD.** Regional differences in fat pad responses to short days in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 257: R1533–R1540, 1989.
4. **Bartness TJ and Wade GN.** Photoperiodic control of seasonal body weight cycles in hamsters. *Neurosci Biobehav Rev* 9: 599–612, 1985.
5. **Blumberg MS, Deaver K, and Kirby RF.** Leptin b disinhibits nonshivering thermogenesis in infants after maternal separation. *Am J Physiol Regul Integr Comp Physiol* 276: R606–R610, 1999.
6. **Dark J, Miller DR, Licht P, and Zucker I.** Glucoprivation counteracts effects of testosterone on daily torpor in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 270: R398–R403, 1996.
7. **Deboer T and Tobler I.** Temperature dependence of EEG frequencies during natural hypothermia. *Brain Res* 670: 153–156, 1995.
8. **Döring H, Schwarzer K, Nuesslein-Hildesheim B, and Schmidt I.** Leptin selectively increases energy expenditure of food-restricted lean mice. *Int J Obes* 22: 83–88, 1998.
9. **Drazen DL, Kriegsfeld LJ, Schneider JE, and Nelson RJ.** Leptin, but not immune function, is linked to reproductive responsiveness to photoperiod. *Am J Physiol Regul Integr Comp Physiol* 278: R1401–R1407, 2000.
10. **Duncan MJ and Goldman BD.** Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, *Phodopus sungorus*. I. Role of the gonads and the pituitary. *J Exp Zool* 230: 89–95, 1984.

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11. **Ebling FJP, Arthurs OJ, Turney BW, and Cronin AS.** Seasonal neuroendocrine rhythms in the male Siberian hamster persist after monosodium glutamate-induced lesions of the arcuate nucleus in the neonatal period. *J Neuroendocrinol* 10: 701–712, 1998.

12. **Gavrilova O, Leon LR, Marcus-Samuels B, Mason MM, Castle AL, Refetoff S, Vinson C, and Reitman ML.** Torpor in mice is induced by both leptin-dependent and -independent mechanisms. *Proc Natl Acad Sci USA* 96: 14623–14628, 1999.

13. **Geiser F, Körtner G, and Schmidt I.** Leptin increases energy expenditure of a marsupial by inhibition of daily torpor. *Am J Physiol Regul Integr Comp Physiol* 275: R1627–R1632, 1998.

14. **Goldman BD, Darrow JM, Duncan MJ, and Yogev L.** Photoperiod, reproductive hormones, and winter torpor in three hamster species. In: *Living in the Cold: Physiological and Biochemical Adaptations*, edited by Heller HC, Wang LCH, and Musacchia XJ. New York: Elsevier, 1986, p. 341–350.

15. **Harris DV, Walker JM, and Berger RJ.** A continuum of slow-wave sleep and shallow torpor in the pocket mouse, *Perognathus longimembris*. *Physiol Zool* 57: 428–434, 1984.

16. **Heldmaier G and Buchberger A.** Source of heat during nonshivering thermogenesis in Djungarian hamsters: a dominant role for brown adipose tissue during cold adaptation. *J Comp Physiol* 156: 237–245, 1985.

17. **Heldmaier G and Steinlechner S.** Seasonal pattern and energetics of short daily torpor in the Siberian hamster, *Phodopus sungorus*. *Oecologia* 48: 265–270, 1981.

18. **Heldmaier G, Steinlechner S, and Rafael J.** Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *J Comp Physiol [B]* 149: 1–9, 1982.

19. **Himms-Hagen J.** Brown adipose tissue metabolism and thermogenesis. *Annu Rev Nutr* 5: 69–94, 1985.

20. **Hoffmann K.** The influence of photoperiod and melatonin on testis size, body weight, and pelage color in the Djungarian hamster (*Phodopus sungorus*). *J Comp Physiol* 85: 267–282, 1973.

21. **Hwa JJ, Fawzi AB, Graziano MP, Ghibaudi L, Williams P, Van Heek M, Davis H, Rudinski M, Sybertz E, and Strader CD.** Leptin increases energy expenditure and selectively promotes fat metabolism in *ob/ob* mice. *Am J Physiol Regul Integr Comp Physiol* 272: R1204–R1209, 1997.

22. **Jolicoeur FB, Boulai SM, Fournier A, and St-Pierre S.** Mapping of hypothalamic sites involved in the effects of NPY on body temperature and food intake. *Brain Res Bull* 36: 125–129, 1995.

23. **Kauffman AS, Cabrera A, and Zucker I.** Torpor characteristics and energy requirements of furless Siberian hamsters. *Physiol Biochem Zool* 74: 876–884, 2001.

24. **Klingenspor M, Dickopp A, Heldmaier G, and Klaus S.** Short photoperiod reduces leptin gene expression in brown and white adipose tissue in Djungarian hamsters. *FEBS Lett* 399: 290–294, 1996.

25. **Klingenspor M, Niggermann H, and Heldmaier G.** Modulation of leptin sensitivity by short photoperiod acclimation in the Djungarian hamster, *Phodopus sungorus*. *J Comp Physiol [B]* 170: 37–43, 2000.

26. **Kotz CM, Briggs JE, Grace MK, Levine AS, and Billington CJ.** Divergence of the feeding and thermogenic pathways influenced by NPY in the hypothalamic PVN of the rat. *Am J Physiol Regul Integr Comp Physiol* 275: R471–R477, 1998.

27. **Kotz CM, Briggs JE, Pomonis JD, Grace MK, Levine AS, and Billington CJ.** Neural site of leptin influence on neuropeptide Y signaling pathways altering feeding and uncoupling protein. *Am J Physiol Regul Integr Comp Physiol* 275: R478–R484, 1998.

28. **Kronfeld-Schor N, Richardson C, Silvia BA, Kunz TH, and Widmaier EP.** Dissociation of leptin secretion and adiposity during prehibernatory fattening in little brown bats. *Am J Physiol Regul Integr Comp Physiol* 279: R1277–R1281, 2000.

29. **Lyman CP, Willis JS, Malan A, and Wang LCH.** *Hibernation and Torpor in Mammals and Birds*. New York: Academic, 1982.

30. **Mercer JG.** Regulation of appetite and body weight in seasonal mammals. *Comp Biochem Physiol* 119C: 295–303, 1998.

31. **Mercer JG, Lawrence CB, Beck B, Burlet A, Atkinson T, and Barrett P.** Hypothalamic NPY and prepro-NPY mRNA in Djungarian hamsters: effects of food deprivation and photoperiod. *Am J Physiol Regul Integr Comp Physiol* 269: R1099–R1106, 1995.

32. **Mercer JG, Moar KM, Ross AW, Hoggard N, and Morgan PJ.** Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. *Am J Physiol Regul Integr Comp Physiol* 278: R271–R281, 2000.

33. **Nicol SC, Pavlides D, and Andersen NA.** Nonshivering thermogenesis in marsupials: absence of thermogenic response to β3-adrenergic agonists. *Comp Biochem Physiol* 117A: 399–405, 1997.

34. **Rafael J, Vsiansky P, and Heldmaier G.** Increased contribution of brown adipose tissue to nonshivering thermogenesis in the Djungarian hamster during cold-adaptation. *J Comp Physiol [B]* 155: 717–722, 1985.

35. **Rayner DV and Trayhurn P.** Regulation of leptin production: sympathetic nervous system interactions. *J Mol Med* 79: 8–20, 2001.

36. **Reddy AB, Cronin AS, Ford H, and Ebling FJP.** Seasonal regulation of food intake and body weight in the male Siberian hamster: studies of hypothalamic orexin (hypocretin), neuropeptide Y (NPY), and pro-opiomelanocortin (POMC). *Eur J Neurosci* 11: 3255–3264, 1999.

37. **Rousseau K, Atcha Z, Cagampang FRA, Le Rouzic P, Stirland JA, Ivanov TR, Ebling FJP, Klingenspor M, and Loudon ASI.** Photoperiodic regulation of leptin resistance in the seasonally breeding Siberian hamster (*Phodopus sungorus*). *Endocrinology* 143: 3083–3095, 2002.

38. **Rousseau K, Atcha Z, and Loudon ASI.** Leptin and seasonal mammals. *J Neuroendocrinol* 15: 409–414, 2003.

39. **Ruby NF.** Paraventricular nucleus ablation disrupts daily torpor in Siberian hamsters. *Brain Res Bull* 37: 193–198, 1995.

40. **Ruby NF, Ibuka N, Barnes BM, and Zucker I.** Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *Am J Physiol Regul Integr Comp Physiol* 257: R210–R215, 1989.

41. **Ruby NF, Nelson RJ, Licht P, and Zucker I.** Prolactin and testosterone inhibit torpor in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 264: R123–R128, 1993.

42. **Ruby NF and Zucker I.** Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 263: R353–R362, 1992.

43. **Ruf T and Heldmaier G.** The impact of daily torpor on energy requirements in the Djungarian hamster, *Phodopus sungorus*. *Physiol Zool* 65: 994–1010, 1992.

44. **Ruf T, Klingenspor M, Pries H, and Heldmaier G.** Daily torpor in the Djungarian hamster (*Phodopus sungorus*): interactions with food intake, activity, and social behavior. *J Comp Physiol B Biochem Syst Environ Physiol* 160: 609–615, 1991.

45. **Ruf T, Steiglitz A, Steinlechner S, Blank JL, and Heldmaier G.** Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (*Phodopus sungorus*). *J Exp Zool* 267: 104–112, 1993.

46. **Scarpace PJ, Matheny M, Pollock BH, and Tumer N.** Leptin increases uncoupling protein expression and energy expenditure. *Am J Physiol Endocrinol Metab* 273: E226–E230, 1997.

47. **Schneider JE, Blum RM, and Wade GN.** Metabolic control of food intake and estrous cycles in Syrian hamsters: I. Plasma insulin and leptin. *Am J Physiol Regul Integr Comp Physiol* 278: R476–R485, 2000.

48. **Stehling O, Döring H, Ertl J, Preibisch G, and Schmidt I.** Leptin reduces juvenile fat stores by altering the circadian cycle of energy expenditure. *Am J Physiol Regul Integr Comp Physiol* 271: R1770–R1774, 1996.

49. **Surmely JF, Voirol MJ, Stefanoni N, Assimacopoulos-Jeannet F, Giacobino JP, Jequier E, Gaillard RC, and Tappy L.** Stimulation by leptin of ^{3}H GDP binding to brown adipose tissue of fasted but not fed rats. *Int J Obes* 22: 923–926, 1998.

50. **Van Dijk G.** The role of leptin in the regulation of energy balance and adiposity. *J Neuroendocrinol* 13: 913–921, 2001.

51. **Walker HC and Romsos DR.** Similar effects of NPY on energy metabolism and on plasma insulin in adrenalectomized *ob/ob* and lean mice. *Am J Physiol Endocrinol Metab* 264: E226–E230, 1993.

52. **Walker JM, Garber A, Berger RJ, and Heller HC.** Sleep and estivation (shallow torpor): continuous processes of energy conservation. *Science* 204: 1098–1100, 1979.

53. **Weiner J.** Limits to energy budget and tactics in energy investments during reproduction in Djungarian hamster (*Phodopus sungorus sungorus* Pallas 1770). *Symp Zool Soc Lond* 57: 167–187, 1987.