Leptin increases energy expenditure of a marsupial by inhibition of daily torpor

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[1]*Zoology, School of Biological Sciences, University of New England, Armidale, New South Wales 2351, Australia; and* [2]*Max-Planck-Institut für physiologische und klinische Forschung, W. G. Kerckhoff-Institut, D-61231, Bad Nauheim, Germany*

Geiser, Fritz, Gerhard Körtner, and Ingrid Schmidt. Leptin increases energy expenditure of a marsupial by inhibition of daily torpor. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R1627–R1632, 1998.—Leptin plays an important role in regulating body fat stores of placental mammals, but the contribution of changes in energy expenditure to this adjustment remains controversial. We were interested in how recombinant murine leptin would affect metabolic rate (MR) and body temperature (T_b) of a marsupial mammal (*Sminthopsis macroura*, 25 g) known to display daily torpor but lacking thermogenetically active brown adipose tissue. In a group of eight animals deprived of food for 1 day at 18°C, leptin treatment halved the duration of torpor bouts (time at $T_b < 30$°C) and raised the average daily minimum T_b by 4.5°C and minimum MR by 2.2-fold. Leptin treatment thus increased daily energy expenditure by 9%, although MR and T_b during the activity phase were not raised. Body mass was also not affected. These findings in a marsupial suggest that the adjustment of thermoregulatory energy expenditure during the rest phase in accordance with energy availability is a phylogenetically old function of leptin.

body temperature; thermoregulation; metabolic rate; *Sminthopsis macroura*

THE HORMONE LEPTIN has primarily attracted attention as an anorectic and slimming substance, potentially useful in treating human obesity (1, 21). It also has been suggested repeatedly that leptin may be involved in the control of energy expenditure (8, 29), although leptin-treated lean rodents with free access to food failed to show any increase in oxygen consumption (13, 22). Moreover, pronounced leptin effects on other hormonal systems observed in starving rodents (1) suggested that a role in the neuroendocrine adaptation to starvation could be another important function or even "the primary purpose for which leptin evolved" (6, 7). Similarly, experiments in early ontogenetic states of the rat, when energy supplies are naturally limited, suggested that disinhibition of sympathetically mediated energy expenditure might be a basic and phylogenetically old function of the hormone, because in these neurally immature animals thermoregulatory thermogenesis, but not food intake, was increased by leptin treatment (27, 28). Related experiments in lean adult mice, which were either free feeding or food restricted, recently confirmed that leptin does not stimulate energy expenditure in general but only disinhibits suppressed thermoregulatory thermogenesis (4). It is possible, however, that this function of leptin may be restricted to animals whose thermoregulatory heat production occurs predominantly in brown adipose tissue (BAT), a tissue specialized for thermogenesis (19), but not present in all endothermic animals. We therefore wondered whether leptin would also adjust energy expenditure in marsupials, which have been separated from placental mammals for over 100 million years and lack thermogenetically active BAT (20).

The nocturnal insectivorous-carnivorous marsupial *Sminthopsis macroura* frequently displays daily torpor, characterized by a controlled reduction of body temperature (T_b) to a minimum of about 15°C and of metabolic rate (MR) to about 30% of the basal MR (9, 11). This strategy is employed by many small mammals and birds to reduce daily energy expenditure during the rest phase, particularly when food is scarce (11). We therefore assessed how recombinant murine leptin affects the diurnal T_b and MR fluctuations as well as the total energy expenditure and body mass in *S. macroura* under experimental conditions conducive to a high occurrence of daily torpor.

MATERIAL AND METHODS

Animals. Eight adult male laboratory-bred *S. macroura* were obtained from a colony at La Trobe University (Melbourne, Australia). For several months before and throughout the experiments animals were maintained individually in cages provided with drinking bottles for free access to water. Food was provided once a day in excess and consisted of a mixture of canned dog food and macerated cat food pellets that was supplemented with calcium and vitamins (12). Animals were acclimated to an ambient temperature (T_a) of 20°C and a photoperiod with lights on from 0200 to 1400. This photophase was selected to permit the control of the equipment for some hours after measurements began at the onset of the activity phase of the animal.

Measurements. To quantify daily fluctuations of T_b and MR, both variables were measured simultaneously over periods of about 23 h. MR was determined by indirect calorimetry. Three animals at a time were placed into separate 0.75-liter respirometry chambers situated within a temperature-controlled cabinet (±0.5°C). The flow rate (about 300 ml/min) of dry air drawn through each of the respirometry chambers was measured with a mass flowmeter (FMA-5606, Omega, Stamford, CT). Oxygen content was measured with a single-channel oxygen analyzer (Ametek Applied Electrochemistry S-3A/1, Pittsburgh, PA) fitted with a high resolution output board (80335SE). The three animal channels and one reference channel were scanned in sequence for 3 min every 12 min with solenoid valves to determine the oxygen content in the outside air and in each of the respirometry chambers. Air

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flow values were corrected to SPTD (0°C, 760 Torr, dry). The rate of oxygen consumption was calculated using *equation 3a* of Withers (31), assuming a respiratory quotient of 0.8, and correspondingly total daily energy expenditure was determined assuming a caloric equivalent of 20.01 kJ/l O_2.

Wax-coated (Parafin/Elvax, Mini-Mitter) temperature-sensitive transmitters (Mini-Mitter, model X-M, accuracy ±0.1°C) were calibrated to the nearest 0.1°C against a mercury thermometer in a water bath between 5 and 40°C. The transmitters were then implanted intraperitoneally under Forthane anesthesia. Animals were allowed to recover for at least 1 wk after operations before measurements began. A ferrite rod antenna underneath each respirometry chamber was used to receive the transmitter signal during measurements of MR. The antennae were multiplexed to a receiver and the transmitter signal transformed to a square-wave signal after background noise was subtracted.

Air temperature in the respirometry chamber was measured to the nearest 0.1°C by a calibrated thermocouple inserted about 1 cm into the chamber. Thermocouple output was amplified by a digital thermometer (Omega DP116).

Analog outputs from the mass flowmeter, oxygen analyzer, transmitter receiver, and digital thermometer were interfaced to a personal computer via a 14-bit analog-to-digital converter card.

Experimental protocol. The animals received, at 1300 for 4 consecutive days, either subcutaneous injections of Tris buffer (*days C1* to *C4*) or recombinant murine leptin (Pepro-Tech, Rocky Hill, 5 μg/g body mass) dissolved in Tris buffer (*days L1* to *L4*). Measurements of MR, T_b, and T_a began immediately after injections and about 1 h before lights off. Because the setup allowed measurements of only three animals per day, the measurements were staggered over 3 days to obtain results for all eight animals. Animals first received control injections for 4 days, and data for each animal were gathered on the 1st and 4th day of control treatment (*C1* and *C4*). For 2 days after the 4th day of control treatment, animals did not receive any injections while measurements on control treatment were completed or leptin treatment began in the other animals. Each animal was thereafter injected for 4 days with leptin, and measurements were performed on the 1st and 4th days of leptin treatment (*L1* and *L4*). Food and water were provided ad libitum when animals were not measured but were withheld during the measurements to increase torpor frequency (9). It has been shown previously that withdrawal of food causes an increase in occurrence of torpor in this species, whereas withdrawal of water alone has no effect (25). Animals were measured at a T_a of 18 ± 1°C, which represents a moderate cold load for this species and results in the absence of food in a >90% occurrence of torpor (9). Animals were weighed before and after each measurement, and a linear mass loss during the day was assumed for calculation of mass-specific MR.

The 23-h measurements were used to calculate torpor bout duration (i.e., the time spent with a T_b below 30.0°C), the frequency at which torpor occurred in the studied population when treated with leptin and buffer (i.e., the proportion of animals that at least once per day reached a T_b below 30.0°C on one of the days of measurement with the same treatment), the daily minimum and maximum T_b and MR (measured over 36 min), the average active T_b and MR (i.e., the average of measurements during which T_b > 30°C during the nightly activity phase), the average daily T_b and the corresponding MR (ADMR, in ml· g^{-1}·h^{-1}), and the total energy expenditure over a day (kJ/day). Body mass and mass loss during days of measurement when food and water were withheld were also determined.

Differences among physiological variables were tested using a one-way repeated-measures ANOVA (*C1* vs. *C4* vs. *L1* vs. *L4*) followed by post hoc comparison of groups (Tukey pairwise comparison). The combined means (of *C1* and *C4* and of *L1* and *L4*, respectively) of data were also compared by a repeated-measures ANOVA. Statistical significance of changes in torpor frequency were evaluated using a χ^2 test. Linear regressions were fitted with the method of least squares. Numerical results are presented as means ± SE for the number of animals measured.

RESULTS

Leptin administration had a pronounced effect on daily fluctuations of T_b and MR of all eight *S. macroura* investigated. As shown for an individual animal that displayed deep and prolonged torpor on both days of measurement with control treatment (*days C1* and *C4*), torpor depth and length were significantly reduced by leptin treatment (Fig.1). This animal reached a T_b as low as 18.9°C during a long torpor bout lasting for about 9 h on *day C4* (Fig. 1*A*). In contrast, torpor was very shallow already after the first leptin injection (*L1*, Fig. 1*B*) with a minimum T_b as high as 28.4°C during a short torpor bout of only about 2 h; a similar shallow and short torpor bout was observed on the 2nd day of measurement with leptin treatment (not shown). In some animals that showed shallow and short torpor during control treatment, leptin prevented torpor entirely.

On average, torpor occurrence was 94% on days of measurement with control treatment and 75% with leptin treatment. Whereas this decline in torpor occurrence was not statistically significant (χ^2 test), torpor duration was significantly reduced during both days of

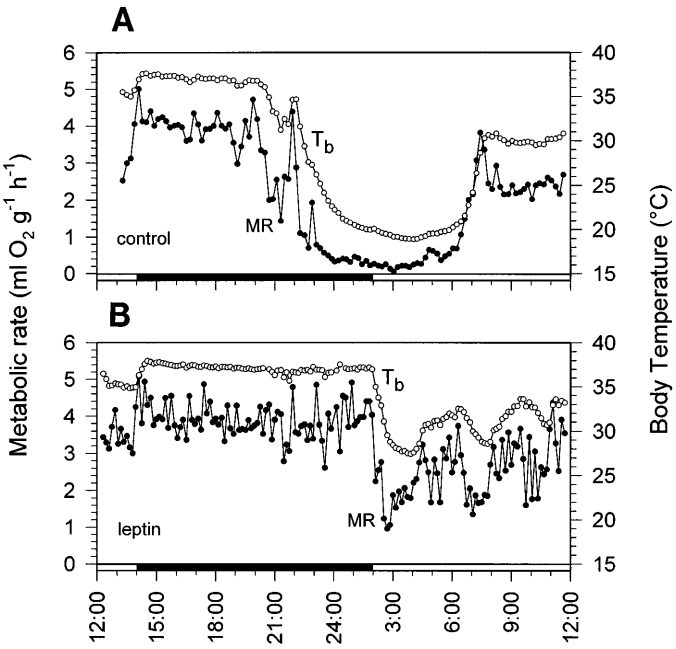

Fig. 1. Example of daily fluctuations of body temperature (T_b) and metabolic rate (MR) in an individual *Sminthopsis macroura* at ambient temperature of 18°C on 4th day (*C4*) of control treatment (*A*) and 2 days later on 1st day (*L1*) of leptin treatment (*B*). Black bar indicates dark phase.

measurement with leptin treatment in comparison to the days of measurement with control treatment ($P < 0.01$, ANOVA). The average duration of torpor bouts on both days of measurement with leptin treatment was about 50% of those with control treatment (Fig. 2*A*). When the last day of measurement with control (*C4*) and leptin (*L4*) treatment were compared, the duration of torpor bouts was reduced to 25%. The reduction of the duration of torpor bouts was associated with an average increase of the daily minimum T_b by 4.5°C (Fig. 2*B*), and the daily minimum MR by 2.2-fold (Fig. 2*C*). Statistical evaluation by repeated-measures ANOVA revealed significant treatment effects ($P < 0.01$) for torpor bout duration, minimum T_b, and minimum MR, as well as for the minimum difference between T_b and T_a (not shown). For each of these variables, post hoc comparison of groups (Tukey at $P < 0.05$) showed significant differences between the last treatment days (*C4* vs. *L4*) but not between the days of measurement with the same treatment (*C1* vs. *C4* and *L1* vs. *L4*). When data obtained for *days 1* and *4* of control and leptin treatments, respectively, were combined and thus only two means for control and leptin treatment were compared, significant treatment effects were observed for torpor bout duration, minimum T_b, minimum T_a, minimum $T_b - T_a$, average daily T_b, and ADMR ($P < 0.025$).

In contrast, maximum T_b and MR were not at all affected ($P > 0.4$, ANOVA) by the leptin treatment (Fig. 3, *A* and *B*). Moreover, average active T_b and MR (i.e., average values at night when $T_b > 30$°C) were not significantly affected by the leptin treatment (Fig. 3, *C* and *D*). This demonstrates that the significant treatment effects found for the average daily T_b (*C4* 32.3 ± 0.8°C vs. *L4* 34.5 ± 0.3°C; $P < 0.01$, ANOVA) and ADMR (*C4* 2.63 ± 0.22 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ vs. *L4* 3.03 ± 0.19 ml $O_2 \cdot g^{-1} \cdot h^{-1}$; $P < 0.01$, ANOVA) was selectively caused by disinhibition of the suppressed thermoregulatory heat production associated with torpor but not by increased metabolism during the nightly activity phase. Meaningful comparisons of mass-specific MR in these experiments were possible because body mass (all means between 24 and 25 g) and loss of body mass (all means between 0.12 and 0.13 g/h) did not show significant treatment effects (ANOVA, $P > 0.5$).

Body mass was not affected by leptin, and MR during activity at night was also constant. Total energy expenditure, however, increased significantly ($P < 0.015$, ANOVA) on the measuring days with leptin treatment (*C4* 29.1 ± 2.8 kJ/day vs. *L4* 34.6 ± 2.4 kJ/day) because torpor was shallower and shorter. On average, total energy expenditure during the 2 days of measurements with leptin was increased by 9% in comparison to that during the 2 days of measurements with control treatment.

Interestingly, the interrelations between the daily minima of physiological variables and the duration of torpor bouts were similar to those in hibernating mammals (10) and did not change with leptin treatment as indicated by strong correlations ($P < 0.001$) of the pooled data obtained from all measurements. The best predictor for the minimum MR was the minimum T_b ($r^2 = 0.85$), whereas the difference between T_b and T_a ($r^2 = 0.69$) explained less of the variance of the daily minimum MR.

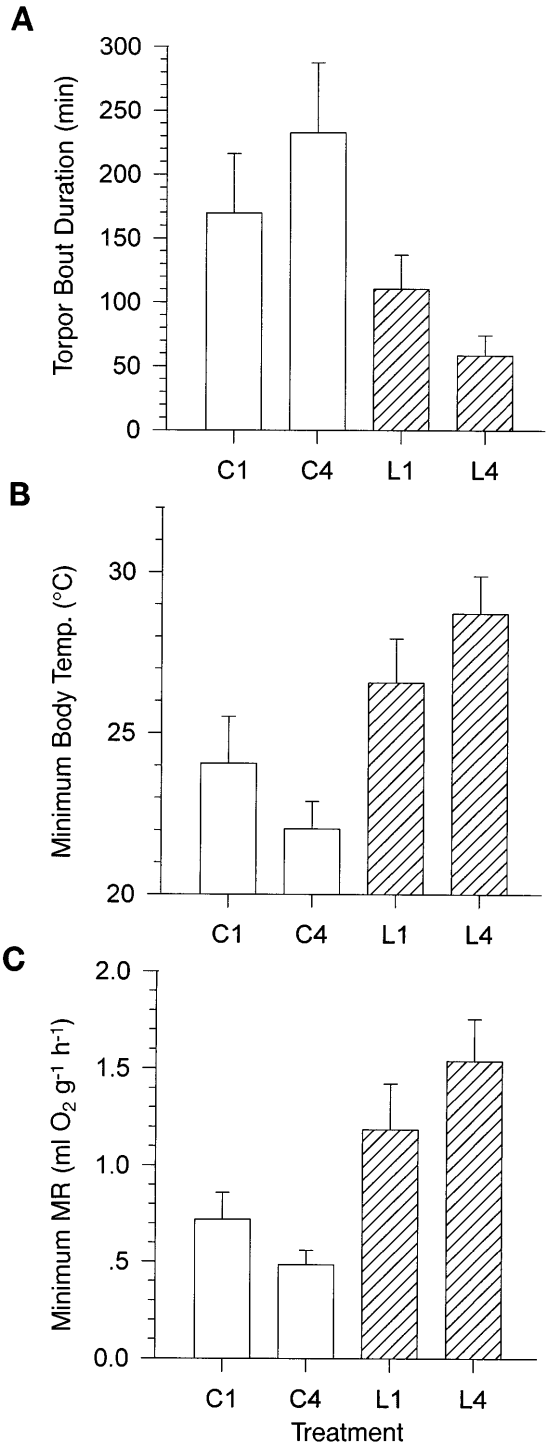

Fig. 2. Effect of leptin on duration of torpor bouts (*A*), daily minimum T_b (*B*), and daily minimum MR (*C*) in *Sminthopsis macroura*. Values are means ± SE for 8 individual animals measured. *C1* and *C4* and *L1* and *L4* show 1st and 4th day of control and leptin treatment, respectively. All values differed significantly among treatments ($P < 0.01$, ANOVA); *C4* and *L4* differed significantly ($P < 0.05$) in post hoc comparisons after ANOVA.

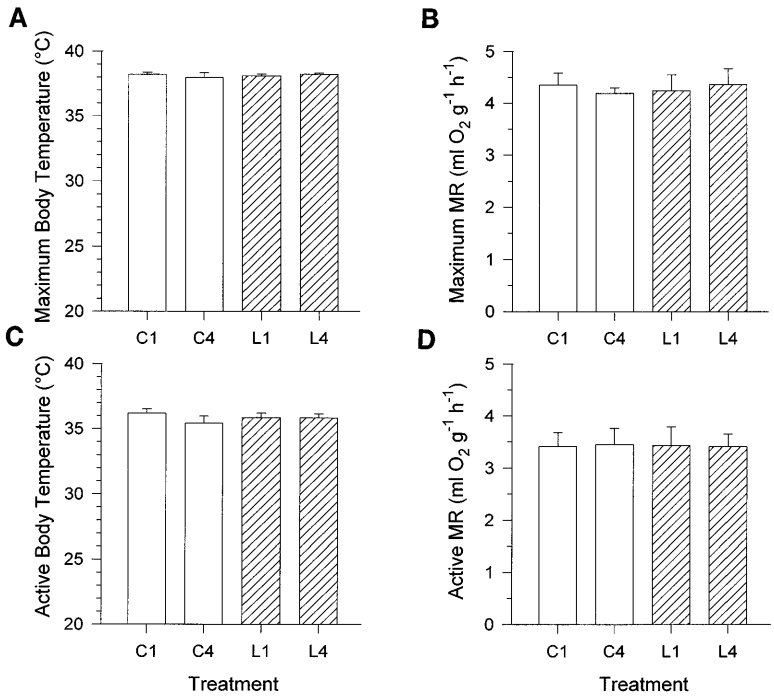

Fig. 3. Effect of leptin on maximum T_b (*A*) and maximum MR (*B*), as well as active T_b (*C*) and active MR (*D*) in *Sminthopsis macroura*. Active T_b and MR are averages of all measurements during the night for which $T_b > 30$°C. Values represent means ± SE for 8 individual animals measured. *C1* and *C4* and *L1* and *L4* show 1st and 4th day of control and leptin treatment, respectively. Variables did not differ among treatments (ANOVA, $P > 0.09$ for active T_b and $P > 0.4$ for other variables).

DISCUSSION

Our study demonstrates that a marsupial mammal, in a condition favoring the occurrence of torpor, responded to leptin treatment by increasing energy expenditure during the rest phase without increasing T_b and MR during the activity phase. These results in a species phylogenetically distant from rodents support and extend previous observations in rats and mice, demonstrating that leptin causes only a disinhibition of reduced MR during torpor or rest (2, 22, 23, 27) and showing that leptin does not cause an increase of MR above its normothermic level (13, 17, 22, 28). The similarity between findings in early ontogenetic states of the placental rat (27) and the present results in marsupials, despite the separation of both groups of mammals in the Cretaceous, endorses the view that the ability of leptin to modulate the energy expenditure for thermoregulation during the rest phase is an early achievement in phylogeny. Moreover, the present findings demonstrate that the presence of functional BAT is no prerequisite for a pronounced leptin effect on overall energy expenditure which is, however, restricted to a disinhibition of sympathetically mediated thermogenesis if it had been suppressed by signals indicating low energy stores.

Although leptin treatment increased total energy expenditure of *S. macroura* by nearly 10% above the values for control treatment, body mass did not change measurably throughout the 4-day treatment period. Therefore, major decreases in food intake of leptin-treated animals on the 2 days between the measurements when the animals had free access to food are unlikely. Unfortunately, captive *S. macroura* tend to scatter their food and thus make reliable determination of differences in food intake difficult. We know, however, from continuous measurements of food intake and MR in mice that a short leptin treatment (3 days) can affect MR without changing food intake and body mass when food availability is restricted (4). As in the present study, the energetic equivalent of the observed changes in MR in food-restricted mice corresponded to changes in body fat stores which were too small (in the range of 0.1 g/day) to change body mass significantly during 3–4 days of leptin treatment, whereas significant changes in body and fat mass could be detected after 10 days of treatment (3, 4). On the other hand, it has been shown in a study on a related marsupial (*S. crassicaudata*) that leptin, as in placental mammals, may also reduce food intake under free-feeding conditions (15). The apparent lack of effect after a 4-day leptin treatment on the body mass of *S. macroura* thus should not be misinterpreted to indicate a diminished response of adipose tissue to leptin in marsupials. In fact, 2 wk of leptin treatment in free-feeding *S. crassicaudata* recently have shown that leptin can affect body mass and fat storage in a marsupial (15).

The strong correlation between the minimum T_b and the minimum MR during torpor raises the question of whether leptin directly affected energy expenditure during the rest phase or whether this effect was indirect via a change of the set point for T_b. At a T_a of 18°C, torpid *S. macroura* are usually well above the set point for T_b during torpor of about 15°C (9, 12, 26), at which cold defense is initiated, and, when in steady-state torpor, usually do not activate thermoregulatory heat production at this T_a. An increase of the set point for T_b, as may be indicated by the 4.5°C rise of the minimum T_b during leptin administration, would require thermoregulatory heat production to increase in torpid individuals to counteract the increased heat loss caused by a larger temperature gradient between the body and the surrounding air and thus explain the

increased minimum MR during torpor. Verification that an increase in the set point for T_b is involved in raising MR during leptin treatment could be achieved by manipulating the hypothalamic temperature and quantifying the resulting metabolic and vasomotor cold defense responses (14).

Perspectives

Our findings that leptin inhibits torpor and thus increases thermogenesis in a marsupial should be considered in light of the vast number of endothermic animals without functional BAT (birds, monotremes, marsupials, and many adult placental mammals). It remains to be established whether similar effects occur in larger animals and humans which show less pronounced daily alterations of energy expenditure, albeit they also decrease their sympathetically mediated thermogenesis when food is restricted (18). It seems therefore important to investigate the possibility that leptin might also counteract the compensatory decrease in energy expenditure during food restriction in species that neither have functionally active BAT nor show torpor.

Previous studies in rodents have shown that leptin, neither when given to animals with very effective BAT (hamsters in winter photoperiod) nor when given to animals with a very low BAT capacity (thermoneutrally reared rat pups), is able to activate thermogenesis under thermoneutral conditions (23, 28). Its function thus differs markedly from that of norepinephrine acting as a peripheral stimulator of thermogenesis. Moreover, the present study shows that the presence of functional BAT is no prerequisite for pronounced leptin effects on energy expenditure. This effect of leptin on the thermogenesis of *S. macroura* was, however, not due to any increase above the normothermic cold- and activity-induced levels of metabolism (see Fig. 3). Although peripheral metabolic effects of leptin might exist (24, 32), our data provide strong evidence against the assumption that leptin effects on thermogenesis are caused by a direct stimulation of any kind of heat-producing mechanisms in BAT or other tissues. We thus hypothesize that leptin also releases suppression of sympathetically mediated heat production in tissues other than BAT. As for other thermogenic mechanisms that might be disinhibited by leptin, we should not only consider shivering but also nonshivering thermogenesis mediated by the recently discovered uncoupling proteins that are not associated with BAT (5, 30). In conclusion, it appears that leptin selectively releases blockage of thermoregulatory energy expenditure by a centrally mediated signal, irrespective of its target.

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