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Leptin disinhibits nonshivering thermogenesis in infants after maternal separation

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Blumberg, Mark S., Kara Deaver, and Robert F. Kirby. Leptin disinhibits nonshivering thermogenesis in infants after maternal separation. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R606–R610, 1999.—Prolonged maternal separation inhibits endogenous heat production in infant mammals exposed to cold. This inhibition of thermogenesis occurs many hours before energy stores have been fully depleted. The need to protect energy resources during separation-induced starvation may be signaled by declining levels of leptin, a hormone that acts as a "fat signal" and a regulator of energy utilization; in fact, starvation reduces leptin levels in adult mice and infant rats. It is not known, however, whether leptin has a functional role during starvation in infants. Such a role may be found in the regulation of nonshivering thermogenesis by brown adipose tissue (BAT), a specialized organ that provides heat to infant mammals, including humans, during cold exposure. Heat produced by BAT allows the cold-exposed infant to prevent the detrimental effects of hypothermia on physiology and behavior and, ultimately, growth. Here we show that leptin disinhibits BAT thermogenesis during cold exposure in infant rats after 18 h of maternal separation. This finding demonstrates that leptin is more than simply an adipostat for the regulation of body weight; specifically, leptin modulates thermogenesis and energy utilization in the early postnatal period.

starvation; thermoregulation; brown adipose tissue; fasting; rat

IN A SERIES OF STUDIES conducted over 20 years ago, Bignall and his colleagues (3–5) examined the effect of acute starvation on heat production during cold exposure in infant rats. In their procedure, infant rats were starved for 1–20 h at a thermoneutral air temperature, after which they were challenged at a subthermoneutral air temperature while oxygen consumption and body temperature were measured. They found that 5-day-old rats challenged at an air temperature of 30°C did not increase metabolic heat production if they were first starved for 10 or more hours (4). Furthermore, this decline in cold-induced thermogenesis in 5-day-old rats was reversed by artificially feeding starved pups with either milk or, to a lesser extent, with glucose or saline.

Bignall and his colleagues (3–5) attempted to determine whether the inhibition of heat production after starvation was due to a depletion of energy stores and/or involved active inhibition by the central nervous system. A subsequent finding that midpontine decerebrations disinhibit heat production even after 10 h of starvation indicates that sufficient energy remains after starvation to support thermogenesis (3). In addition, midpontine decerebration also maintained high blood glucose levels in starved pups, suggesting that glucoreceptors may modulate the onset of thermogenesis. Subsequent experiments, however, failed to provide support for the hypothesis that blood glucose levels provide a controlling signal for the stimulation of nonshivering thermogenesis (5).

In recent years, the discovery of leptin, a hormone secreted by fat cells and thought to act as a signal of fat stores, has provided new insights into the interrelations between energy regulation, obesity, and brown adipose tissue (BAT) thermogenesis (13, 15, 18). One popular current model posits that fat cells produce and secrete leptin in proportion to fat stores, and leptin then acts within the hypothalamus to suppress food intake and to burn off excess fat by activating BAT thermogenesis. According to this model, strains of mice that either lack the gene that is responsible for the production of leptin (*ob*/*ob*) or leptin receptors (*db*/*db*) become obese because they overeat and BAT thermogenesis cannot be recruited to help regulate fat levels. Moreover, leptin dysregulation during infancy results in altered thermoregulation in response to cold exposure; this has been shown in infant Zucker (*fa*/*fa*) rats that, like *db*/*db* mice, are insensitive to leptin (14, 16). Indeed, obesity in Zucker rats can be prevented by chronic and selective pharmacological activation of BAT thermogenesis during infancy (9).

Unlike adults, infants are not well-served by wasting excess energy; for infants, energy derived from maternal nutrition is best directed toward growth. When exposed to cold temperatures, however, many mammalian infants activate BAT thermogenesis and, by doing so, counteract successfully the detrimental effects of hypothermia on various behavioral and physiological responses (6). For example, in cold-exposed infant rats, warmed blood exiting the interscapular BAT deposit flows back to the heart where, by warming cardiac tissue directly, it helps to maintain cardiac rate in the

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cold (7, 20). Therefore, for infants, BAT functions to provide heat, not waste, energy.

The modulation of BAT thermogenesis by leptin in adults and the thermoregulatory function of BAT in infants suggests the hypothesis that leptin mediates the starvation-induced inhibition of heat production investigated by Bignall and his colleagues (3–5). Support for this hypothesis comes from the observation that starvation leads to a reduction in leptin levels in infant rats (10), just as it does in adult mice (1, 2). Therefore, we examine here whether administration of leptin disinhibits BAT thermogenesis in 4- to 5-day-old rats starved for 18 h.

METHODS

Subjects. Thirty-two male and female offspring from eight litters, 4–5 days of age at the beginning of the experiment, were used. Pups were born to Harlan Sprague-Dawley females maintained in the animal colony at the University of Iowa. Mothers and their litters were housed in standard laboratory cages (48 × 20 × 26 cm) in which food and water were available ad libitum. Litters were culled to eight pups within 3 days after birth (day of birth = *day 0*). All animals were maintained on a 12:12-h light-dark schedule with lights on at 0600.

Apparatus and physiological measures. Detailed descriptions of the apparatus and the methods used to measure physiological temperatures and oxygen consumption can be found elsewhere (7, 8). Briefly, experiments were conducted by placing pups inside a double-walled glass metabolic chamber. By pumping temperature-controlled water through the walls of the chamber, air temperature was controlled. Inside the chamber, pups remained unrestrained on top of a polyethylene mesh platform.

Air temperature (T_a) and physiological temperatures were measured using chromel-constantan thermocouples. T_a was measured using a thermocouple placed beneath the mesh platform. For measurements of physiological temperature, thermocouples were attached to the dorsal skin surface of the subject using collodion as an adhesive. One thermocouple was attached in the interscapular region directly above the BAT deposit and provided a measure of interscapular temperature (T_{is}). A second thermocouple was attached ~1 cm rostral to the base of the tail; this thermocouple provided a measure of skin temperature (T_{back}) distant from the site of heat production.

For the measurement of oxygen consumption, compressed air passed through a regulator and was divided into two lines. One line passed through a flowmeter, and the air in the line was then humidified and circulated through the metabolic chamber at 300 ml/min. The air was drawn from the chamber and desiccated, after which it was drawn through one of two channels of an electrochemical oxygen analyzer. The second line of air flowed directly from the regulator to the second channel of the oxygen analyzer. The oxygen content of each airstream was measured simultaneously, and the percent difference in concentration was computed to within 0.001%. On the basis of the airflow rate through the chamber and the pup's body weight, the percent oxygen difference was transformed into oxygen consumption ($\dot{V}O_2$) in milliliters O_2 per kilogram per minute.

Procedure. Pups were separated from their mother and littermates on postnatal *day 4* or *5*, weighed, and placed in an incubator maintained at an air temperature of 34–36°C and a relative humidity >60%. Sixteen hours after separation and 2 h before cold challenge, each of three littermates was weighed again and either *1*) injected with a volume of sterile isotonic saline equal to the amount of body weight lost over the preceding 16 h, *2*) injected with a weight-replacing volume of sterile isotonic saline in which murine recombinant leptin (ICN Biomedicals) was dissolved to provide a final dose of 1 μg/g body wt, or *3*) given a sham injection. A fourth littermate was neither starved nor injected. The dose of leptin chosen for this study was based on previous work demonstrating the time course for circulating levels of leptin and the presence of sympathoexcitation after peripheral administration (1, 11).

The four littermates were tested over 2 days (2 pups on each day) and were assigned to different conditions using a balanced design. Pairs of pups were tested 4 h apart beginning in the morning, and groups were counterbalanced to control for time of day.

At least 1 h before cold challenge began, the two thermocouples were attached and the pup was transferred from the incubator to the temperature-controlled metabolic chamber. During acclimation, T_a within the chamber was maintained at 35°C. At the time corresponding to 17 h and 50 min after maternal separation, acquisition of thermal and metabolic data began; data were recorded by the data acquisition system at a rate of 4 samples/min. Then, after 10 min of baseline data acquisition (i.e., after 18 h of maternal separation), air temperature was decreased to 31°C and remained there for 45 min. Next, air temperature was decreased in succession to 27.5 and 23.5°C, with 45 min of data collection at each air temperature. After the test, the pup was removed from the chamber and returned to its home cage.

Data analysis. Data were imported into StatView 4.5 for the Macintosh, and the thermal and metabolic data at the end of baseline and the end of each 45-min period of air temperature decrease were selected. Data were analyzed using repeated-measures ANOVA. Paired *t*-tests were used for post hoc comparisons between groups at each air temperature. α was set at 0.05, and the Bonferroni procedure was used to correct α for multiple comparisons. All data are presented as means ± SE.

RESULTS

Separating infant rats from maternal care for 16 h resulted in 0.4–0.5 g reductions in body weight (Table 1). After 18 h of starvation in a thermoneutral environment, T_{is}, T_{back}, and $\dot{V}O_2$ were significantly decreased in all three groups of pups relative to the unstarved controls. This generalized suppression of metabolism at thermoneutral air temperature was not affected by leptin administration and may have been due to declining levels of thyroxine (1). Regardless, to standardize the comparisons of variables for each pup at each air temperature, subsequent data are presented as change from baseline values.

Prior starvation for 18 h resulted in significant reductions in T_{is} from baseline at all three subthermoneutral air temperatures in relation to unstarved controls (Fig. 1*A*). Greater cooling by the starved pups was apparently not due to dehydration, as isotonic saline injections that replaced the lost volume had no effect. Pups injected with murine recombinant leptin, however, exhibited values of T_{is} that were higher than the other two groups of starved pups, although not as high as the unstarved controls.

Table 1. *Effects of starvation on body weight, skin temperatures, and oxygen consumption*

	Unstarved	Starved + Saline + Leptin	Starved + Saline	Starved + Sham Injection
Body wt, g				
Before starvation		11.8 ± 0.4	11.4 ± 0.4	11.4 ± 0.4
After starvation (day of test)	12.7 ± 0.7	11.4 ± 0.4	10.9 ± 0.4	11.0 ± 0.4
T_{is} at baseline, °C	37.7 ± 0.1	36.2 ± 0.03*	36.2 ± 0.1*	36.1 ± 0.1*
T_{back} at baseline, °C	37.4 ± 0.1	36.0 ± 0.03*	36.0 ± 0.1*	35.9 ± 0.1*
$\dot{V}O_2$ at baseline, $ml \cdot kg^{-1} \cdot min^{-1}$	3.76 ± 0.18	2.51 ± 0.09*	2.46 ± 0.10*	2.45 ± 0.10*

Values are means ± SE; $n = 7$ or 8 per group. Male and female 4 to 5-day-old rats were isolated overnight in an incubator maintained at an air temperature of 34–36°C with relative humidity >60%. Body weight was measured before starvation (i.e., at 4–5 days of age) and after 16 h of starvation (i.e., at 5–6 days of age) in the 3 experimental groups. For the unstarved group, body weight was measured only on the day of testing (i.e., at 5–6 days of age). Baseline thermal and metabolic measures were recorded at an air temperature of 35°C after 18 h of starvation. T_{is}, interscapular temperature; T_{back}, lumbar temperature; $\dot{V}O_2$, O_2 consumption. $P < 0.0001$ for thermal and metabolic measures using ANOVA; *$P < 0.017$ (paired *t*-test) compared with unstarved pups.

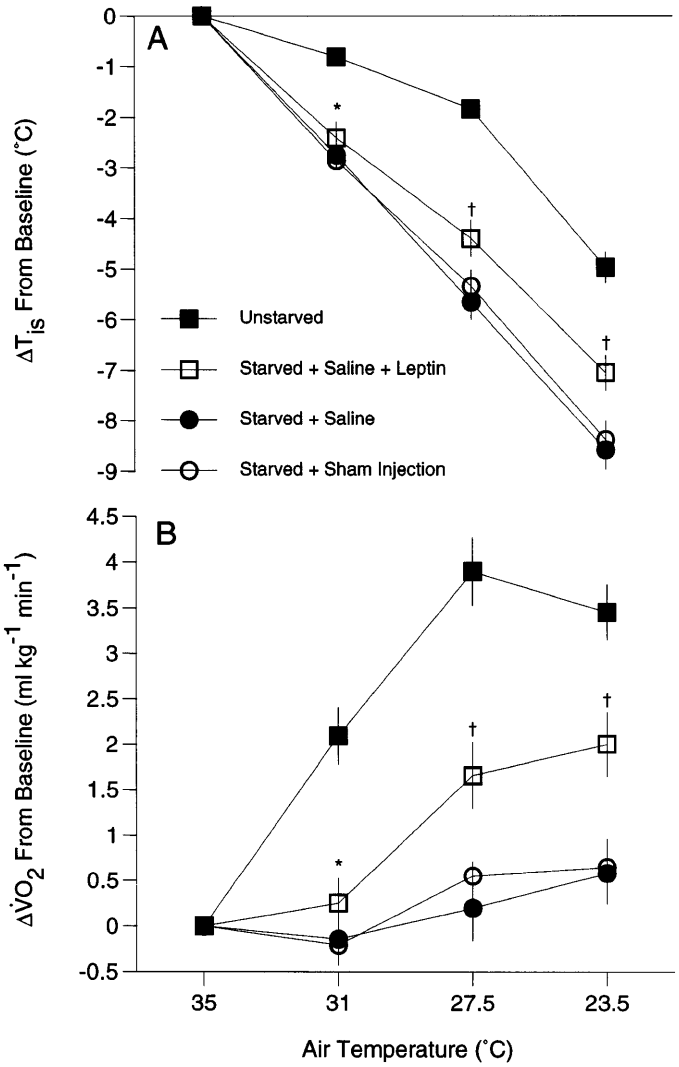

Fig. 1. Leptin disinhibits heat production in starved rat pups during cold exposure. *A*: change in interscapular temperature (ΔT_{is}) from baseline values (air temperature 35°C) at successively lower air temperatures for each of 4 experimental groups. *B*: change in oxygen consumption ($\Delta\dot{V}O_2$) from baseline values. $n = 7$ or 8 rats/group. All main effects and interactions, $P < 0.0001$ by repeated-measures ANOVA. *$P < 0.017$ (paired *t*-test) compared with unstarved pups; †$P < 0.017$ (paired *t*-test) compared with other 3 experimental groups.

Because 1-wk-old rats cannot produce heat by shivering (22), nonshivering thermogenesis by BAT is the primary means of endogenous heat production during cold exposure. The largest deposit of BAT is in the interscapular region (19), and T_{is} reflects heat production at its source (Fig. 2). To conclude that BAT thermogenesis has been activated, however, it is also necessary to detect increases in $\dot{V}O_2$. As expected from our previous work (6, 8), unstarved control pups in-

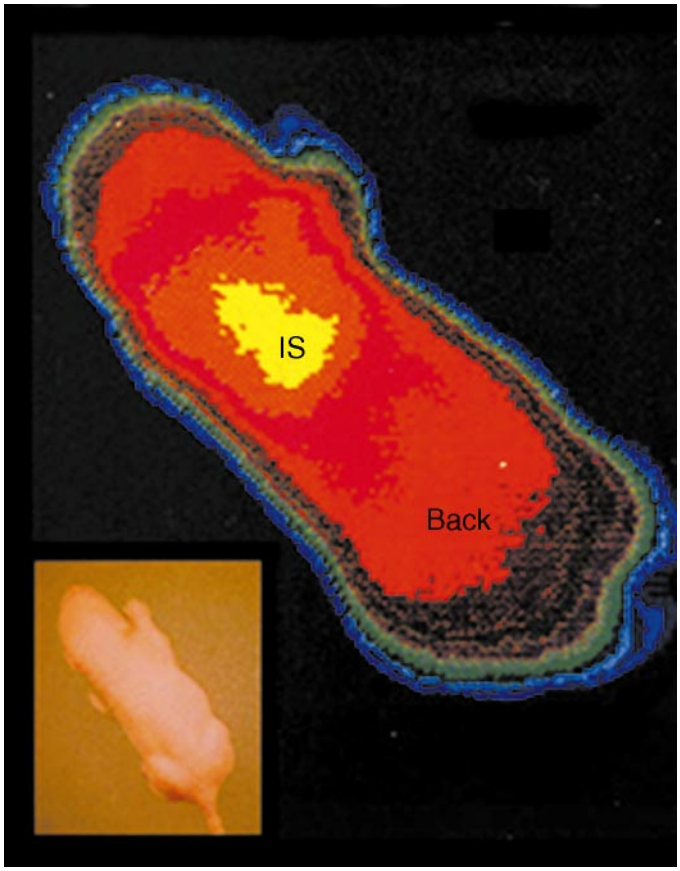

Fig. 2. Infrared thermograph showing brown adipose tissue (BAT) thermogenesis in an infant rat during cold exposure. A conventional photograph of a similarly oriented pup is shown *bottom left*. Yellow area indicates a "hot zone" overlying large interscapular (IS) deposit of brown fat. (In contrast, thermographs of infant rats in a thermoneutral environment present homogeneous temperature distributions.) In the present study, thermocouples attached in IS and lumbar areas (Back) provided estimates of BAT thermogenesis.

creased $\dot{V}O_2$ as air temperature decreased (Fig. 1*B*). These increases were significantly greater than the modest increases exhibited by the starved pups that were either sham injected or injected with saline. Pups injected with leptin, however, exhibited increases in $\dot{V}O_2$ that were significantly greater than those of the other starved pups.

In addition to increases in $\dot{V}O_2$ during cold exposure, increases in the differential between T_{is} and T_{back} strengthen the conclusion that interscapular BAT thermogenesis has been activated (9; Fig. 2). Thus at the thermoneutral air temperature T_{is} and T_{back} were nearly equivalent in each experimental group, reflecting the absence of BAT thermogenesis (Table 1). In contrast, as air temperature decreased and $\dot{V}O_2$ increased, the differentials between T_{is} and T_{back} increased in all experimental groups, reflecting increased heat production in the interscapular region. These differentials ranged from 0.6 to 1.5 and 1.0 to 1.9°C at air temperatures of 27 and 23°C, respectively, with group rankings corresponding to the $\dot{V}O_2$ data in Fig. 1*B*.

DISCUSSION

The thermal and metabolic responses of the starved pups injected with leptin all point to a disinhibition of BAT thermogenesis during cold exposure. Although substantial, this disinhibition was not sufficient to return pups to the unstarved state; of course, it is possible that larger doses of leptin would have increased this effect. Regardless of the dose of leptin used, however, complete reversal of the effects of starvation on nonshivering thermogenesis was unlikely in this experiment given the extreme length of starvation employed (i.e., 18 h) and the consequent diminished energy resources available for thermogenesis. In this regard it should be stressed that as few as 10 h of starvation are sufficient to suppress thermogenesis by 5-day-old rats in the cold (4).

Although leptin disinhibits nonshivering thermogenesis in starved pups, the exact sites of heat production have not been determined. The increasing differential between interscapular and lumbar temperatures during cold exposure in leptin-treated pups suggests activation of thermogenesis in the interscapular BAT, the largest deposit of BAT in infant rats (19). It is also likely, however, that nonshivering thermogenesis was activated in other BAT deposits located throughout the body.

The current, dominant model of leptin's functional significance posits that when fat cells are replete with triglycerides and leptin is being secreted in high amounts, leptin then acts within the hypothalamus to suppress food intake and increase energy utilization (via BAT thermogenesis) as a means of depleting excess fat and regulating body weight (24). Support for this model has been provided by investigations of both mutant and nonmutant rodent strains (12, 15, 17, 23). In these studies, however, heat production is viewed as a byproduct of BAT's function as a fat-burning organ and, although perhaps appropriate within the context of the adult, this view ignores BAT's initial function as a vital source of heat for the infant (6). In contrast, our results suggest that high levels of leptin, perhaps resulting from the high-fat diet of the suckling infant (10), provide a permissive environment for the activation of BAT thermogenesis during cold exposure and, when leptin levels decline during maternal separation, energy is conserved by inhibiting BAT thermogenesis in the cold. Thus the view of leptin as an energy-regulating hormone applies equally to infants and adults (21), although our results indicate a much broader function for leptin than simply as a regulator of fat stores. Furthermore, our results imply that the metabolic role of BAT thermogenesis in the adult emerges from its thermoregulatory role in the infant. Therefore, leptin appears to modulate energy use throughout life in a variety of contexts, beginning in the first week postpartum as a mediator in situations where the infant's needs for energy conservation and use are in conflict.

Perspectives

We are currently witnessing an explosion of interest in the role of leptin and other hormones in the regulation of food intake, energy utilization, and fat storage (24). Thus far most of this interest has focused on the functional role of leptin in adults and has led quickly to a consensus regarding this hormone's primary role in the regulation of body weight. Studies in infant animals can broaden our perspective of a system's organization by unveiling its developmental origins. Thus, although the present results support the notion that leptin modulates energy use and fat storage via its effects on BAT thermogenesis, this modulatory influence on BAT begins within a thermoregulatory context at a time during development when the deposition of fat is a primary concern.

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