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IMMEDIATE COMMUNICATION

Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa

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Semi-starvation induced hyperactivity (SIH) occurs in rodents upon caloric restriction. We hypothesized that SIH is triggered by the decline in leptin secretion associated with food restriction. To test this hypothesis, rats, which had established a stable level of activity, were treated with leptin or vehicle via implanted minipumps concomitantly to initiation of food restriction for 7 days. In a second experiment treatment was initiated after SIH had already set in. In contrast to the vehicle-treated rats, which increased their baseline activity level by 300%, the development of SIH was suppressed by leptin. Furthermore, leptin was able to stop SIH, after it had set in. These results underscore the assumed major role of leptin in the adaptation to semi-starvation. Because SIH has been viewed as a model for anorexia nervosa, we also assessed subjective ratings of motor restlessness in 30 patients with this eating disorder in the emaciated state associated with hypoleptinemia and after increments in leptin secretion brought upon by therapeutically induced weight gain. Hypoleptinemic patients ranked their motor restlessness higher than upon attainment of their maximal leptin level during inpatient treatment. Thus, hypoleptinemia might also contribute to the hyperactivity frequently associated with anorexia nervosa. *Molecular Psychiatry* (2000) **5,** 476–481.

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Hyperactivity is observed in 40–80% of patients with anorexia nervosa (AN).[1,2] Caloric deprivation severe enough to result in significant weight loss possibly provokes sensations of behavioral arousal and activation in individuals with an innate vulnerability to develop AN.[3] Indeed, reduced food intake and enhanced activity have been viewed as the core symptomatology in AN, because only these behavioral measures consistently distinguish AN from other disorders.[4]

Semi-starvation induced hyperactivity (SIH) is a well characterized phenomenon in laboratory animals.[5,6] Rats supplied with food for only 1 h per day manage to survive, but die within a short period of time when exposed to a running wheel. The enhancement of activity is related to the severity of food restriction; total food deprivation results in a disruption of the nocturnal activity pattern.[6]

Leptin is assumed to be the major hormone underlying the regulatory phenomena that lead to an adaptation of an organism to reduced energy supplies.[7] Thus, in mice exogenously applied leptin has been shown to blunt the semi-starvation-induced downregulation of the hypothalamic-pituitary gonadal and thyroid axes as well as the upregulation of the hypothalamic-pituitary adrenal axis.[7] The rapid decline in leptin secretion associated with caloric restriction and weight loss[7,8] could represent the initial trigger underlying the hyperactivity observed in SIH and AN. As an initial step towards testing this hypothesis we investigated the effect of continuous leptin treatment via minipumps on SIH in rats. In parallel, we describe the subjective experience of motor restlessness in 30 patients with AN, both during initial hypoleptinemia and after weight gain at times of maximal leptin secretion during inpatient treatment.

In the first series of animal studies, a total of 30 rats which had previously established a stable activity

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level, were subjected to a 7-day long continuous infusion of either leptin ($n = 15$) or vehicle ($n = 15$; controls) via subcutaneously implanted minipumps. After implantations an equal number of leptin-treated and control rats were allocated either to an ad libitum diet ($n = 16$) or a food restriction amounting to 60% of the previous ad libitum intake ($n = 14$), respectively. Implantations, irrespective of treatment, caused an intermittent decrease of activity (Figure 1a and b).

Running wheel activity amongst the seven food-restricted controls bounced back to the pre-surgery level within 1 day (Figure 1a). During the following week food restriction continuously promoted the development of SIH up to a mean maximum of 4800 revolutions on day 7 corresponding to 300% of the pre-surgery baseline. In marked contrast and in accordance with our hypothesis, the activity of the seven food-restricted rats infused with leptin only gradually increased after the implantation, but even at day 7 did not significantly exceed the presurgery baseline. In ad libitum-fed animals, leptin treatment apparently facilitated the post-surgery recovery to baseline activity lev-

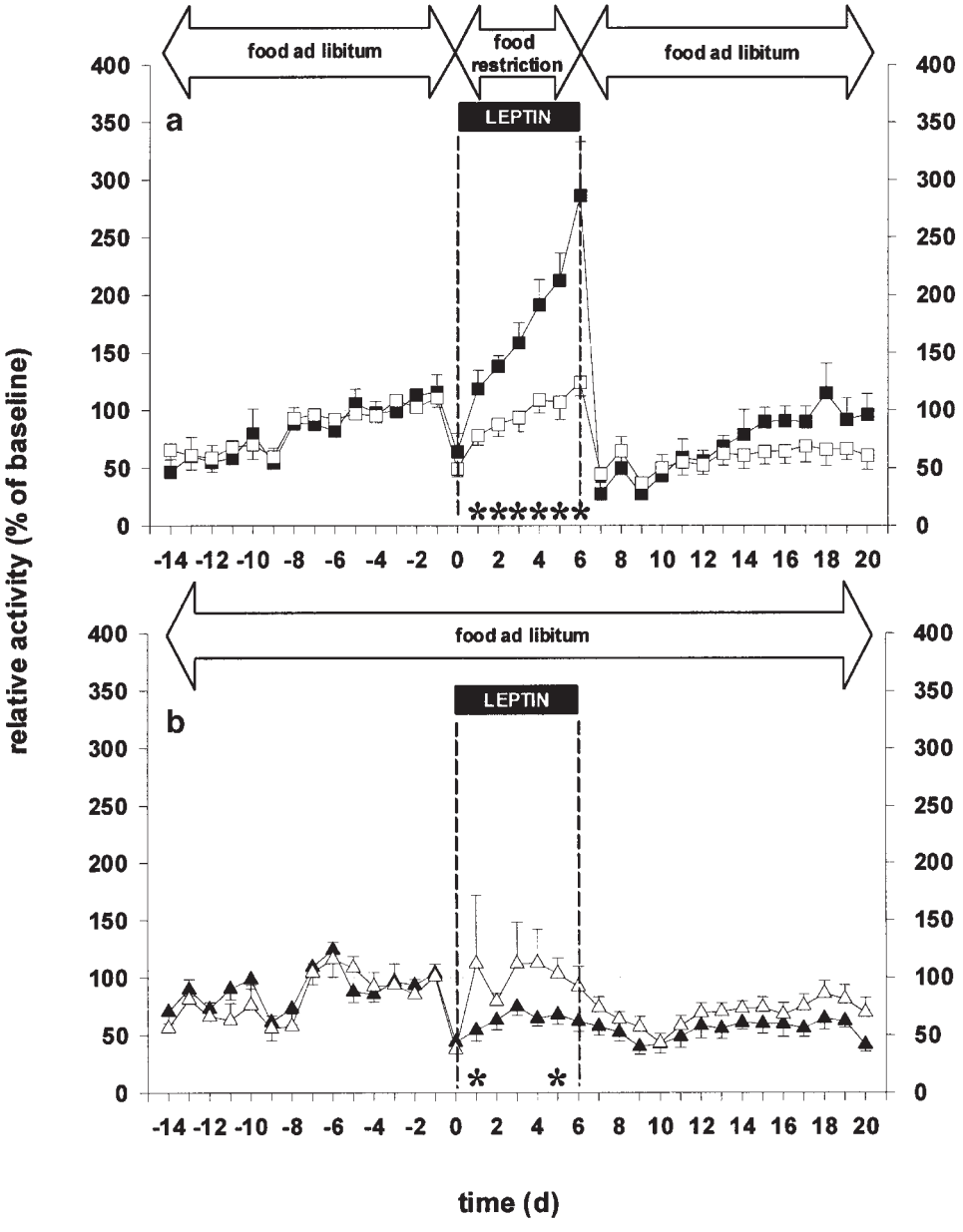

Figure 1 Suppression of semi-starvation induced hyperactivity (SIH) by leptin. Running wheel activity was recorded in rats, which were either restricted to 60% of their ad libitum food intake for 1 week (a, $n = 7$ in each treatment group) or had free access to food (b, $n = 8$ in each treatment group). Rats were treated with leptin (open symbols) or vehicle (closed symbols) via implanted minipumps beginning on the first day of food restriction. The duration of leptin application is indicated by the black bars and the period of food restriction is highlighted by the shaded arrow. The recording of running wheel activity started 2 weeks prior to treatment. Activity is presented as a percentage ± SEM of mean activity during the second pretreatment week, to adjust for individual variation in baseline activity. Asterisks denote a significant effect of leptin treatment on activity ($P < 0.05$).

els as compared to controls (Figure 1b), which might be related to the previously detected stress-reducing effect of leptin in rodents.[9]

Irrespective of mode of treatment, all food-restricted rats displayed a clear nocturnal pattern of activity with only 7–11% of total activity occurring during the light phase. In addition to the inhibitory effect on SIH, leptin treatment also altered the diurnal activity pattern during initial refeeding, during which minipumps no longer delivered leptin. Thus, in rats previously treated with leptin, daytime activity remained low, whereas in control animals the nocturnal activity pattern was disrupted during the first 3 days of refeeding, with daytime activity increasing by 27–44%. On the first day of refeeding, leptin-treated rats consumed less food as compared to controls (28.4 ± 1.4 g *vs* 34.7 ± 1.9 g; $P < 0.05$). Possibly the strong refeeding response in controls caused an altered circadian activity pattern, which was not observed in the leptin-treated animals, perhaps as a consequence of a lowered foraging drive.

In accordance with previous results in other rodents,[10,11] leptin treatment of ad libitum-fed rats caused reduced weight gain (5.8 ± 2.3 g per 7 days *vs* 23.6 ± 2.2 g per 7 days, $P < 0.001$) and reduced food intake (19.5 ± 0.78 g day^{-1} *vs* 22.37 ± 0.93 g day^{-1}; $P < 0.05$) in comparison to controls. Surprisingly, despite the large difference in locomotor activity, hyperactive controls did not lose more body weight during semi-starvation for 1 week than leptin-treated rats (mean body weights ± SE at postsurgery day 7: 250.2 ± 8.2 g *vs* 252.4 ± 10.5 g). This could possibly be explained by findings in rodents that exogenous leptin blunts the semi-starvation-induced decrease of resting metabolic rate.[12,13]

A second experiment was designed to test whether leptin treatment can 'rescue' hyperactive rats after SIH has set in. After food restriction for 5 days had induced SIH in all 14 rats, implantations of the pumps again led to a transient drop in activity. As expected, the seven control rats again increased activity during the following week of continued food restriction. In marked contrast, activity levels of the seven leptin-treated rats only slowly increased after implantations, but did not exceed the levels observed prior to food restriction (Figure 2). Measurements of serum leptin levels upon termination of the experiment 7 days after implantations revealed an approximately two-fold elevation in the leptin-treated rats as compared to controls (1406 ± 161 pg ml^{-1} *vs* 745 ± 151 pg ml^{-1}, $P < 0.05$), thus indicating that minipumps were still releasing leptin.

Serum leptin levels rapidly decrease in response to semi-starvation[7,8] and in addition, exercise in itself potentially may contribute to hypoleptinemia.[14] Our results clearly suggest that the decrease of serum leptin levels in food-restricted rats is the major signal which triggers the expression of SIH, thus further extending the assumed key role of this hormone in semi-starvation[7] to the behavioral level. A prolonged reduction of caloric intake in humans and rodents is accompanied by several adaptive mechanisms which counterregulate the development of negative energy balance. In this context, SIH appears to be a paradoxical reaction. However, if SIH leads to increased foraging behavior in a free-ranging animal, this may enhance fitness and can be viewed as a successful strategy to cope with limited energy resources.

Physical activity and reduced food intake have previously been invoked to increase hypothalamic neuropeptide Y (NPY) levels[15] and to activate the hypothalamic-pituitary-adrenal axis. Elevated cortisol levels may sensitize the dopaminergic and noradrenergic neurons of the limbic system and reinforce self-starvation via reward mechanisms.[4] Very recently it was demonstrated that leptin can modulate the brain reward circuitry.[16] Leptin affects neuropeptidergic systems controlling energy metabolism including orexic NPY and anorexic corticotropin releasing factor (CRF). Leptin inhibits the semi-starvation-induced rise in NPY synthesis[17] and prevents the stimulation of CRF synthesis and the activation of the adrenal axis.[18] We hypothesize that the inhibitory action of exogenous leptin on SIH is most likely to be mediated through these hormones.

In an initial attempt to address the relationship between serum leptin levels and hyperactivity in patients with AN, the subjective experiences of motor restlessness in 30 patients (26 females, 4 males) with AN were assessed at three time points during therapeutically induced weight gain (Figure 3): (a) upon admission for inpatient treatment (T_0) in the emaciated state associated with hypoleptinemia (0.65 ± 0.56 μg l^{-1}; range: 0.015–1.62 μg l^{-1}); (b) after serum leptin levels (2.38 ± 1.22 μg l^{-1}) for the first time surpassed the 25th percentile of the gender-dependent reference range formed by healthy controls matched for BMI[19]; and (c) upon attainment of maximal leptin levels (T_{max}) measured during inpatient treatment (5.86 ± 3.85 μg l^{-1}; range 1.20–14.60 μg l^{-1}). After leptin levels for the first time topped the 25th percentile of the reference range, patients reported a trend towards reduced motor restlessness ($P = 0.13$; one-sided) in comparison to baseline. Upon attainment of the maximal leptin levels motor restlessness was ranked lower as compared to baseline ($P = 0.023$; one-sided).

The higher subjective ratings of motor restlessness associated with hypoleptinemia upon initiation of treatment, in comparison to lower ratings observed upon attainment of maximal levels, need to be interpreted cautiously. This reduction could be caused by any one or a combination of several factors other than the increment in leptin secretion. Because the ranking of motor restlessness can theoretically also imply a subjective feeling with no overt behavior, objective measurements of energy expenditure and physical activity in parallel to monitoring of leptin secretion are clearly required to verify that motor activity indeed decreases. Nevertheless, our results in both rodents and humans are readily compatible with the hypothesis that hypoleptinemia contributes to the hyperactivity observed in patients with AN.

Whereas SIH has been discussed as a model for the

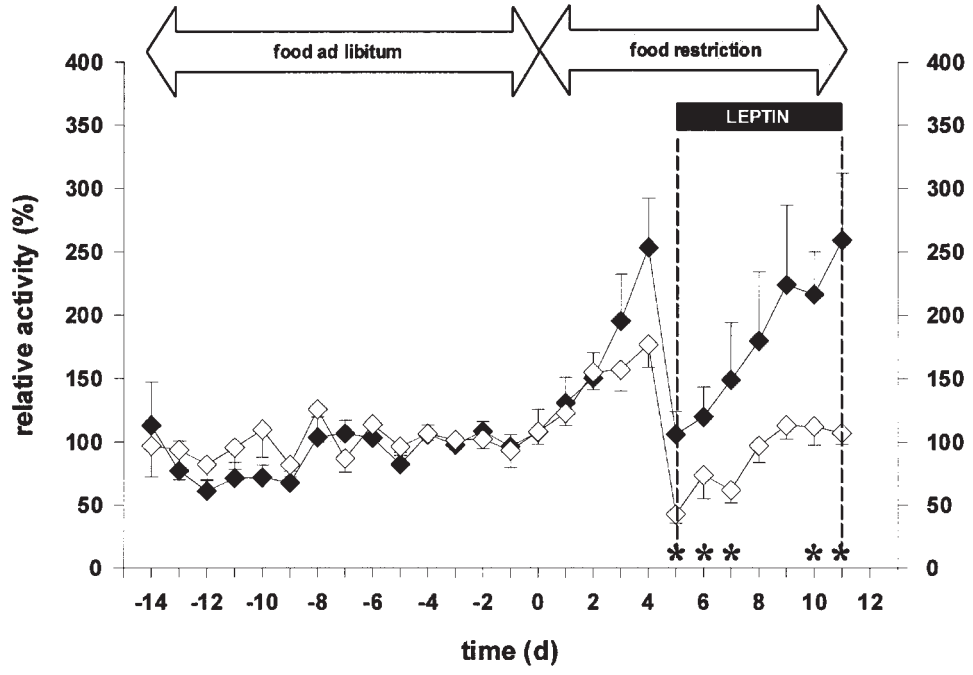

Figure 2 Leptin inhibition of running wheel activity after semi-starvation induced hyperactivity (SIH) had set in. Rats were food restricted for 12 days to 60% of the ad libitum food intake, beginning 5 days prior to the start of either leptin (open symbols) or vehicle (closed symbols) treatment via implanted minipumps ($n = 7$ in each treatment group). The duration of leptin application is indicated by the black bar and the period of food restriction is highlighted by the shaded arrow. Activity is presented as a percentage ± SEM of mean activity during the week before the beginning of food restriction, to adjust for individual variation in baseline activity. Asterisks denote a significant effect of leptin treatment on activity ($P < 0.05$).

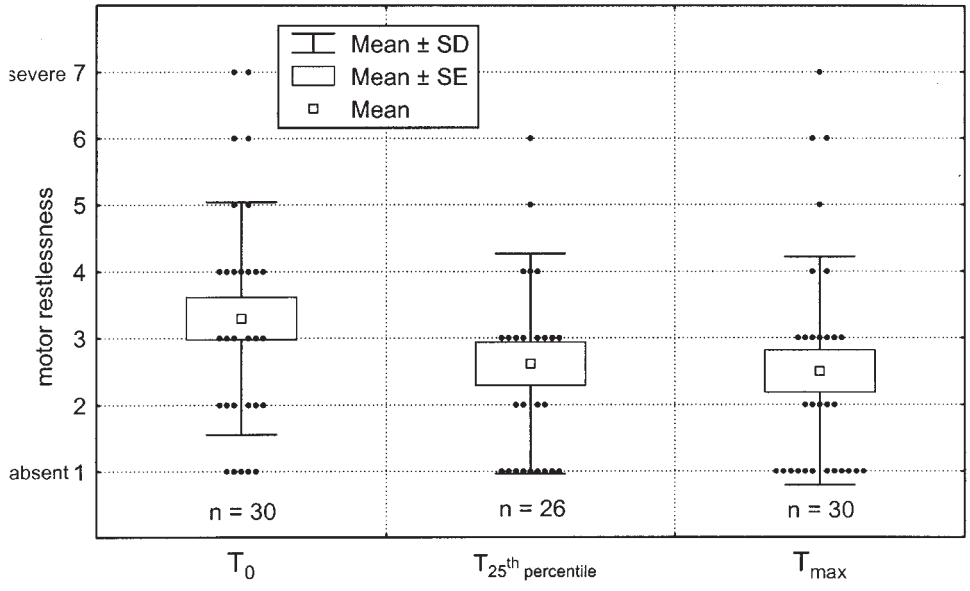

Figure 3 Subjective ratings of motor restlessness in 30 patients (four males) with anorexia nervosa at three time points during therapeutically induced weight gain: (1) upon admission for inpatient treatment in the emaciated state (T_O); (2) upon attainment of the first serum leptin level at or greater than the 25th percentile of the reference range formed by healthy controls matched for gender and body mass index[23] (T_{25th}; four patients had already had a leptin level above this percentile at admission); and (3) upon attainment of the maximal leptin level during inpatient treatment (T_{max}). Serum leptin levels were determined biweekly throughout treatment.

hyperactivity observed in AN,[4] other investigators have been concerned with obvious differences.[20] Thus, AN in comparison with SIH is associated with a prolonged reduction in food intake. In clinical terms the hyperactivity in some patients seems to be strongly motivated by their pursuit of thinness. However, in other patients the hyperactivity appears beyond the patient's control, thus potentially representing a more biologically driven form of hyperactivity. In our opinion, especially this latter form of hyperactivity could be driven by hypoleptinemia.

In AN, therapeutically induced weight gain leads to normalization of leptin secretion; during this process leptin levels can even intermittently exceed the reference range formed by BMI and gender-matched healthy controls.[19,23] Indeed, in 13 of the 30 patients included in the present study the maximal leptin level exceeded the 90th percentile of the reference range. Patients with AN are well suited to analyse the potential implications of both hypoleptinemia upon emaciation and the increment in leptin secretion during weight gain. Because leptin normalizes activity levels in food-restricted rats, leptin administration in acute AN can possibly reduce hyperactivity potentially rendering patients more amenable to treatment.

Materials and methods

Male Wistar 'HsdCpb. WU' rats (Harland Laboratories, Germany) at an age of 38 days, weighing 140–163 g, were housed in individual cages equipped with a running wheel (circumference 110 cm) in a climate chamber (23 ± 1°C). Lights were on from 6:00 am to 6:00 pm. Rats intitially had free access to rat chow (R/M-H, sniff Spezialdiäten GmbH, Germany) and water. On-line activity monitoring started at the age of 52 days, when average body mass had reached 232 g. Body weight and food intake were continuously monitored at 5:00 pm in 2–3 day intervals.

In two independent experiments, food-restricted or ad libitum-fed rats were treated with either recombinant mouse leptin (1.29 mg ml^{-1}, supplied by Hoechst AG, Frankfurt, Germany) or with vehicle (phosphate-buffered saline, PBS) via subcutaneously implanted miniosmotic pumps (Alzet, Model 2001), which release the respective fluids at a rate of 1 $\mu l\ h^{-1}$ for 7 days according to the manufacturer. Thus, rats received 31 μg recombinant mouse leptin per day.

For the first series of experiments a total of 30 rats were allocated to two equally sized vehicle- and leptin-treated groups, by matching for pretreatment activity and body weight. Within each treatment group rats were fed ad libitum ($n = 8$) or restricted to 60% of ad libitum food intake ($n = 7$) during the 7-day treatment period and (re)fed ad libitum during 14 days of post-treatment. Minipumps were removed 10 days after implantation. In the second experiment 14 rats were food restricted for 12 days to 60% of ad libitum food intake, with leptin treatment via implantation of minipumps starting on day 6. To verify ongoing leptin infusion at the end of the experiment (7 days after implantation), serum leptin levels were assayed as described previously[19] in both leptin-treated and control rats.

The effect of leptin on running wheel activity was analyzed by a repeated-measures ANCOVA procedure adjusted for the activity level of individual rats 1 week before treatment (SPSS 8.0). For graphical presentation running wheel activity was expressed as a percentage of the mean activity during the week prior to food restriction. Mean serum leptin levels in control and leptin-treated rats were compared by Student's *t*-test. Data are presented as mean ± SEM.

For the purpose of our study we selected 30 patients (4 males) with AN (DSM-IV criteria; mean age ± SD: 16.44 ± 3.5 years; range: 12.3–30.7), who presented with hypoleptinemia (initial serum leptin levels $<1.85\ \mu g\ l^{-1}$)[24] and whose serum leptin levels during therapeutically induced weight gain topped the 25th percentile of the reference range formed by healthy gender and BMI-matched controls.[19,23] The 30 patients were blood sampled at 8:00 am within 3 days after admission for inpatient treatment to allow determination of serum leptin levels and thereafter biweekly until discharge as reported previously.[23] Their mean BMI (± SD) upon the initial sampling after admission was $14.50 \pm 1.36\ kg\ m^{-2}$ (range: 11.87–16.80 $kg\ m^{-2}$). All patients and in case of minors their respective parents gave written informed consent for participation in the clinical study, which was approved by the Ethics Committee of the University of Marburg.

Concomitantly to the biweekly blood samplings patients were asked to rate their subjective experience of motor restlessness during the preceding week on a seven-point visual analogue scale with a score of 1 indicating no motor restlessness and a score of 7 indicating severe restlessness. The relationship between the patients' serum leptin levels and their scores for motor restlessness were compared at three time points: (1) upon admission in the semi-starved condition associated with hypoleptinemia; (2) after initial normalisation of serum leptin levels determined on an individual basis as the first leptin level achieved during weight gain that topped the 25th percentile of the reference range; and (3) finally after attainment of the individual maximal leptin level determined biweekly during inpatient treatment. Upon attainment of these maximal leptin levels the patients' mean BMI (± SD) had increased to $17.39 \pm 1.02\ kg\ m^{-2}$; range: 15.66–19.14 $kg\ m^{-2}$). We hypothesized that patients would rate their subjective experience of motor restlessness higher upon admission.

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