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Role of leptin in the neuroendocrine response to fasting

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A TOTAL deficiency in or resistance to the protein leptin causes severe obesity[1–4]. As leptin levels rise with increasing adiposity in rodents[5] and man[6,7], it is proposed to act as a negative feedback 'adipostatic signal' to brain centres controlling energy homeostasis, limiting obesity in times of nutritional abundance[1,3]. Starvation is also a threat to homeostasis that triggers adaptive responses[8–12], but whether leptin plays a role in the physiology of starvation is unknown. Leptin concentration falls during starvation[13] and totally leptin-deficient *ob/ob* mice have neuroendocrine abnormalities similar to those of starvation[14], suggesting that this may be the case. Here we show that preventing the starvation-induced fall in leptin with exogenous leptin substantially blunts the changes in gonadal, adrenal and thyroid axes in male mice, and prevents the starvation-induced delay in ovulation in female mice. In contrast, leptin repletion during this period of starvation has little or no effect on body weight, blood glucose or ketones. We propose that regulation of the neuroendocrine system during starvation could be the main physiological role of leptin.

Depriving male mice of food for 48 h caused a 16% fall in weight (Table 1) and reduced circulating leptin from 3.87 ± 0.35 to 1.5 ± 0.11 ng ml^{-1} (Fig. 1*a*). Blood glucose concentration fell from 159 to 63.4 mg dl^{-1}, insulin levels fell from 1.23 to 0.18 ng ml^{-1} and β-hydroxybutyrate rose from 2.85 to 19.3 mg dl^{-1} (Table 1). Recombinant leptin injected intraperitoneally (i.p.) into fasted mice produced peak serum concentrations after 1 h, had an apparent half-life of 3 h, and concentrations 6 and 12 h after injection were similar to fed controls (Fig. 1*b*). When fasted mice were given twice-daily i.p. injections of leptin (1 μg per g body weight), circulating levels after 48 h were similar to fed controls (Fig. 1*a*), and leptin repletion did not alter weight loss or levels of glucose, insulin or β-hydroxybutyrate (Table 1). On recommencing feeding, the compensatory rise in food intake was marked during the first 6 h (Table 1). Leptin injection caused a reduction in food intake over the first 4 h of refeeding, and weight gain after 24 h (Table 1).

Starvation disrupts fertility in humans, non-human primates and rodents[11,15,16], most probably at the level of the hypothalamus, as starvation decreases gonadotropin pulse frequency, and levels of gonadotropins and sex steroids[15]. We characterized the effect of leptin treatment on the oestrus cycle of mice. Food deprivation for 48 h prolonged dioestrus in all saline-treated mice and delayed vaginal oestrus by 6.3 ± 1.3 days (Fig. 2*a*), despite 98% of body weight being regained within 24 h of recommencing feeding. In contrast, 7 of 10 leptin-treated fasted mice had no delay in onset of oestrus, and dioestrus was prolonged by 1–3 days in 3 others (Fig. 2*a*). As in males, leptin did not alter weight loss during starvation, but decreased the rate of weight gain for 24 h after refeeding (data not shown). We found equivalent effects in males, where 48 h of fasting caused an 88% decrease in serum testosterone concentrations from 1.09 ± 0.18 to 0.12 ± 0.02 ng ml^{-1} (Table 2). Treatment with leptin decreased the fall to 58% of control levels. Serum luteinizing hormone was measurable in 8 of 13 fed mice (1.29 ± 0.40 ng ml^{-1}) and undetectable in the other 5. Luteinizing hormone was undetectable in all 13 fasted mice, and leptin treatment restored levels to nearly 40% of those in fed controls in the 6 of 13 mice where it was detectable (Table 2), indicating that leptin acts at the level of the hypothalamic–pituitary system.

The changes in adrenal and thyroid axes induced by starvation are also influenced by leptin. Serum thyroxine decreased from 0.85 ± 0.05 to 0.18 ± 0.02 μg dl^{-1} with fasting, and leptin

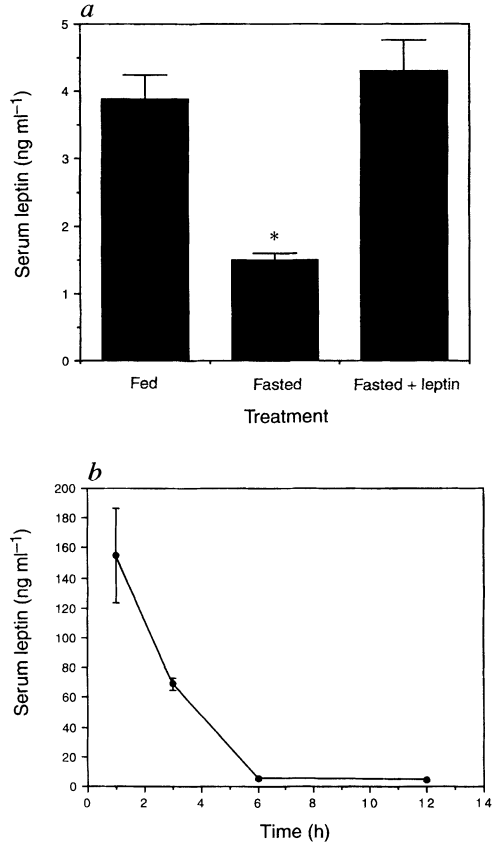

FIG. 1 *a*, Serum leptin was measured in fed, fasted and fasted + leptin-treated mice (see Table 1 for details) with a radioimmunoassay kit to mouse leptin (Linco Research Inc.). Data are means ± s.e.m., $n = 12$ per group. *$P < 0.05$ by ANOVA and Fisher PSLD. *b*, Kinetics of intraperitoneal (IP) recombinant leptin in fasted mice. Serum leptin was measured 1, 3, 6 and 12 h after i.p. injection of recombinant mouse leptin into mice fasted for 48 h; $n = 5$ per group.

TABLE 1 Effects of fasting and leptin treatment

	Fed	Fasted	Fasted + leptin
Body weight (g)			
Initial	24.5 ± 0.5	24.8 ± 0.4	24.7 ± 0.3
After fasting	24.9 ± 0.3	20.7 ± 0.4*	20.3 ± 0.3*
Refeeding 24 h	24.7 ± 0.5	23.8 ± 0.5	22.8 ± 0.3
48 h	24.6 ± 0.7	24.9 ± 0.4	24.8 ± 0.5
Change in weight after 24 h refeeding (g)	0.2 ± 0.1	3.2 ± 0.1*	2.6 ± 0.2*†
Blood glucose (mg dl^{-1})	159 ± 5.5	63.4 ± 1.1*	61.1 ± 2.6*
β-hydroxybutyrate (mg dl^{-1})	2.85 ± 0.35	19.3 ± 2.52*	17.2 ± 1.54*
Plasma insulin (ng ml^{-1})	1.23 ± 0.34	0.18 ± 0.03*	0.20 ± 0.05*
Cumulative food intake during refeeding (g chow per mouse)			
2 h	0.39 ± 0.01	1.06 ± 0.05*	1.00 ± 0.05*
4 h	1.20 ± 0.01	1.85 ± 0.05*	1.64 ± 0.03*†
6 h	1.52 ± 0.03	2.32 ± 0.05*	2.43 ± 0.06*
12 h	3.06 ± 0.12	3.83 ± 0.05*	3.82 ± 0.04*
24 h	3.77 ± 0.18	4.50 ± 0.08*	4.43 ± 0.10*
48 h	7.28 ± 0.38	9.26 ± 0.06*	9.20 ± 0.07*

Male C57BL mice (Jackson Laboratory), were housed in plastic cages under constant environmental conditions, a 12-h dark (18:00–06:00h) and 12-h light (06:00–18:00h) cycle, allowed free access to chow and water, and mice were handled twice daily at 09:00 and 18:00 h. At 7 weeks of age, the mice were divided into three groups. After recording body weight and food intake during the dark and light cycles, one group was allowed continued access to chow, the second was deprived of chow for 48 h and received twice daily intraperitoneal (IP) injections of recombinant mouse leptin (Eli Lilly), 1 μg per g body weight, at 09:00 and 18:00 h, and third was fasted for 48 h and treated with saline. Twelve h after the last dose of leptin, the mice were killed by decapitation and truncal blood obtained for measurement of blood glucose and plasma β-hydroxybutyrate with enzyme assays (Sigma), and insulin by radioimmunoassay (Linco Research). Data are means ± s.e.m., n = 10–13 per group. To determine the effect of leptin treatment on refeeding, the weight of chow consumed by fasted mice was measured 2, 4, 6, 12, 24 and 48 h after the last dose of leptin or saline vehicle at 18:00 h (at the beginning of the dark cycle). Data are means ± s.e.m., n = 12 per group. *P < 0.05 compared with fed mice; †P < 0.05 compared with fasted mice by ANOVA and Fisher PSLD.

decreased the fall in thyroxine to 0.42 ± 0.05 μg dl^{-1} (Table 2). Mean plasma corticosterone and adrenocorticotropic hormone (ACTH) in fed mice, measured at 11:00 h during the light cycle, were 162 ± 25 ng ml^{-1} and 46.7 ± 4.7 pg ml^{-1} respectively, and fasting increased corticosterone to 340 ± 24 ng ml^{-1} and ACTH to 103 ± 10.8 pg ml^{-1} (Table 2). Leptin blunted the rise in corticosterone to 238 ± 27 ng ml^{-1} and ACTH to 47.3 ± 5.1 pg ml^{-1}. Leptin may therefore be involved in mediating the diurnal pattern of the hypothalamic–pituitary–adrenal (HPA) axis.

A diurnal variation of circulating leptin was seen in male mice fed *ad libitum* (Fig. 2*b*). Leptin concentration was 3.35 ± 0.62 ng ml^{-1} at 08:00h, and declined during the light cycle, reaching a nadir early in the dark cycle at 20:00 h. Concentration then increased during the dark cycle, coincident with the period of maximum food intake, and reached a peak of 4.2 ± 0.08 ng ml^{-1} at 04:00h at the end of the dark cycle. There was a reciprocal temporal relationship between circulating leptin and corticosterone. Serum corticosterone was lowest (46.3 ± 9.7 ng ml^{-1}) at the end of the dark cycle, and increased during the light cycle, peaking at 172.3 ± 31.8 ng ml^{-1} at 24:00h during the dark cycle. A role of leptin in regulating the diurnal pattern of corticosterone in mice is suggested.

The effects of leptin suggest regulation at the level of the hypothalamus. Leptin receptors have been localized to the choroid plexus and hypothalamus[17,18] and neuropeptide Y (NPY) is a potential mediator of its central effects[4]. Hypothalamic NPY increases with starvation[19], and intracerebroventricular NPY inhibits gonadotropins and sex steroids[20] and activates the HPA axis[9]. The amount of hypothalamic NPY messenger RNA doubled after 48 h of starvation, and was decreased 12 h after leptin treatment (Table 2), consistent with the idea that effects of starvation are mediated by leptin regulation of NPY. A similar role for NPY has been implicated in the action of leptin in reversing the phenotype of *ob/ob* mice, which also exhibit reproductive failure and increased corticosterone levels responsive to chronic leptin treatment[4,21]. Although leptin suppressed NPY and exerted neuroendocrine actions during starvation, its effect on recommencement of feeding was minimal, suggesting that factors independent of NPY play a role in compensatory feeding after starvation, as suggested by observations in NPY-knockout mice[22].

Several of the responses to starvation were only partially reduced by leptin. Although leptin levels in treated animals

TABLE 2 Effect of fasting and leptin treatment on hormone levels and hypothalamic NPY mRNA

	Fed	Fasted	Fasted + leptin
Testosterone (ng ml^{-1})	1.09 ± 0.18	0.12 ± 0.02*	0.45 ± 0.04*†
LH (ng ml^{-1})	1.29 ± 0.40	< 0.36	0.52 ± 0.07*†
Thyroxine (μg dl^{-1})	0.85 ± 0.05	0.18 ± 0.02*	0.42 ± 0.05*†
Corticosterone (ng ml^{-1})	162 ± 24.8	340 ± 24.4*	238 ± 27.1*†
ACTH (pg ml^{-1})	46.7 ± 4.7	103 ± 10.8*	47.3 ± 5.1†
NPY mRNA (arbitrary densitometric units)	9.5 ± 0.8	19.5 ± 2.1*	12.9 ± 1.6†

Effect of fasting and recombinant mouse leptin on pituitary–gonadal and pituitary–adrenal hormones and thyroxine. See Table 1 for details of treatment. Testosterone and thyroxine were measured by radioimmunoassay (DPC, Los Angeles), serum luteinizing hormone (LH) by a modification of the rat LH radioimmunoassay using mouse standards[23]. Plasma ACTH and corticosterone were measured by radioimmunoassay kits (DPC and ICN Pharmaceuticals, respectively) in mice killed at 11:00 h during the light cycle. Data are means ± s.e.m., n = 10–13 per group for testosterone, ACTH, corticosterone and thyroxine. LH was detectable (>0.36 ng ml^{-1}) by single determination in 8 of 13 fed mice, undetectable in all 13 fasted saline-treated mice, and dectable in 6 of 13 fasted + leptin-treated mice. Total RNA was prepared from individual hypothalami, n = 3 per group, and analysed by northern blot hybridization with a complementary RNA probe to mouse NPY as described previously[24]. *P < 0.05 compared with fed controls; †P < 0.05 compared with fasted mice by ANOVA and Fisher PSLD for testosterone, corticosterone, ACTH and thyroxine; For LH, *P < 0.05 (t-test) compared with fed controls.

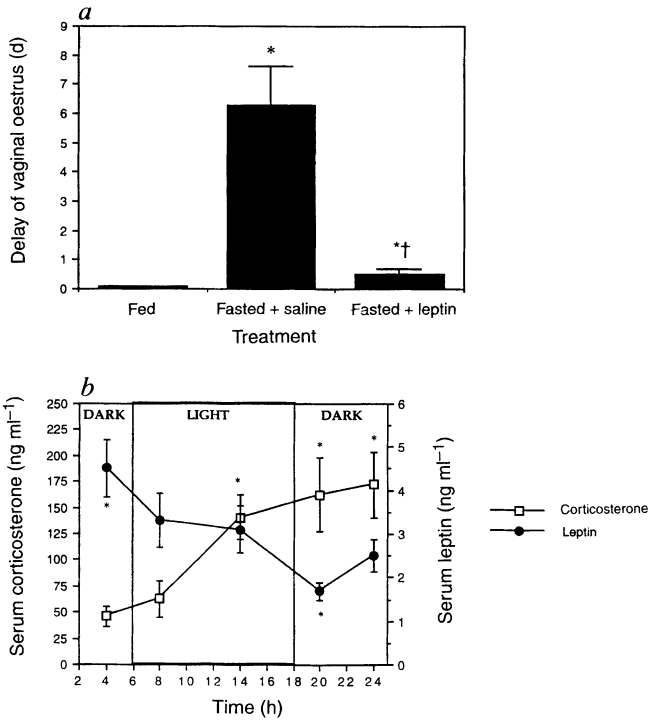

FIG. 2 *a*, Effect of fasting and leptin treatment on the oestrus cycle. Female C57BJ mice were housed singly in plastic cages under ambient conditions, with a 12 h light (06:00–1800) and 12 h dark (18:00–06:00) cycle, and free access to chow and water. Daily vaginal smears were obtained from age 8 weeks for the duration of 3 oestrus cycles. Thirty females with regular 4–5 day oestrus cycles were assigned to 3 treatment groups (10 per group). One group was fed *ad libitum*, and the others were fasted for 48 h during dioestrus, and treated with twice daily i.p. injections of recombinant mouse leptin, 1 μg per g body weight or saline. Body weight decreased by 15%, from 23.0 ± 0.9 to 19.6 ± 0.9 gm, and leptin treatment did not alter this. Twelve h after the last injection, all mice were allowed free access to food. Body weight was regained to 98% of control after 24 hours and fully restored after 48 hours. Daily vaginal smears were obtained, each female serving as its own control. The delay of vaginal oestrus was determined as the difference between the length of the cycle (oestrus to oestrus) before and after treatment[11]. Data are means ± s.e.m., $n = 10$ per group. *$P < 0.05$ compared with fed controls; †$P < 0.05$ compared with fasted mice by ANOVA and Fisher PSLD. *b*, Diurnal variation of serum leptin and corticosterone. Male C57BL mice were used (Table 1), and handling was restricted to cage cleaning. Seventy per cent of food intake (2.78 ± 0.18 g chow per mouse) occurred during the dark cycle, and 30% (1.19 ± 0.10 g chow per mouse) during the light cycle. Groups of mice ($n = 5$) were killed by decapitation at 04:00, 08:00, 14:00, 20:00 and 24:00 h. Serum corticosterone and leptin were measured by radioimmunoassay (ICN and Linco, respectively). Data are means ± s.e.m., *$P < 0.05$ compared with 08:00 h by ANOVA and Fisher PSLD.

were similar to those of fed controls, recombinant leptin may be less potent, or the leptin radioimmunoassay may overestimate bioactive leptin. Alternatively, as falling insulin may mediate adaptation to starvation through regulation of hypothalamic NPY[9], leptin and insulin may cooperate to regulate aspects of the neuroendocrine response to starvation.

In an environment where periodic limitations of food availability, rather than continuous access, is common, the ability to adapt to starvation is fundamentally important to survival of the species. These studies show that falling leptin concentration is a critical signal that initiates the neuroendocrine response to starvation, including limiting procreation, decreasing thyroid thermogenesis, and increasing secretion of stress steroids, which together are likely to have survival value during prolonged nutritional deprivation. Given the high prevalence of apparent leptin resistance in obese rodents[5,6] and humans[6,7], the physiological response to decreasing leptin concentration with starvation may be the dominant role of this hormone. □

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Consolidation in human motor memory

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LEARNING a motor skill sets in motion neural processes that continue to evolve after practice has ended, a phenomenon known as consolidation[1–4]. Here we present psychophysical evidence for this, and show that consolidation of a motor skill was disrupted when a second motor task was learned immediately after the first. There was no disruption if four hours elapsed between learning the two motor skills, with consolidation occurring gradually over this period. Previous studies in humans and other primates have found this time-dependent disruption of consolidation only in explicit memory tasks[5–12], which rely on brain structures in the medial temporal lobe[9,13,14]. Our results indicate that motor memories, which do not depend on the medial temporal lobe[8,15], can be transformed by a similar process of consolidation. By extending the phenomenon of consolidation to motor memory, our results indicate that distinct neural systems share similar characteristics when encoding and storing new information.

Subjects moved the handle of a two-link planar manipulandum[16] (Fig. 1*a*) to guide a cursor to a series of 192 targets (one target set) that appeared one at a time on a computer monitor mounted above the manipulandum (Fig. 1*b*). On the first day of testing (day 1), after baseline trajectories were recorded (Fig. 1*b*),

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