

EXPERIMENTAL STUDY

Leukemia inhibitory factor reduces body fat mass in ovariectomized mice

John-Olov Jansson, Sofia Movérare-Skrtic, Anna Berndtsson, Ingrid Wernstedt, Hans Carlsten¹ and Claes Ohlsson
Research Center for Endocrinology and Metabolism and ¹Department of Rheumatology and Inflammation Research, Department of Internal Medicine, University of Göteborg, Sweden

(Correspondence should be addressed to J-O Jansson, Department of Physiology/Endocrine Section, Medicinargatan 9A, SE413 45 Göteborg, Sweden; Email: JOJ@medic.gu.se)

Abstract

Objective: Ovariectomized (OVX) mice are known to gain body fat while exposure to estrogens decreases fat mass. We have previously shown that estrogen replacement therapy enhances the expression of receptors for the cytokine, leukemia inhibitory factor (LIF). LIF and other cytokines acting via the gp130 signal transducing receptor have been reported to decrease obesity. In the present study, we investigated whether LIF treatment can reduce obesity in OVX mice.

Design: Eight-week-old female C57Bl/6 mice were OVX or sham-operated. The mice were treated with LIF, 30 µg/kg or PBS via daily i.p. injections for 15 days ($n = 9-10$).

Methods: Dual X-ray absorptiometry and computerized tomography.

Results: We found that LIF treatment of OVX mice caused a significant reduction in the weight of white fat depots ($P = 0.017$) and serum leptin levels ($P = 0.011$). LIF also caused a significant decrease in brown fat mass ($P = 0.036$). Treatment with LIF decreased thymus weight but did not affect crown-rump length, femur length, trabecular bone mineral density or the weight of several non-fat organs including the uterus.

Conclusion: The cytokine, LIF, decreases body fat mass in OVX mice, suggesting that estrogen signaling is not required for this effect.

European Journal of Endocrinology 154 349–354

Introduction

The prevalence of obesity is growing worldwide. This is a major health problem as it is well known that obesity, especially visceral obesity, is associated with increased risk of, for example, type II diabetes, hypertension, and cardiovascular disease (1). Unfortunately, at present there are few effective therapies for obesity (2). One of the most successful routes for identification of body fat regulating mechanisms and thereby possible drug targets in humans has been the investigation of genes shown to affect body fat mass in mice (3, 4).

Estrogens are well known suppressors of visceral fat in both animals and humans (5–8), but the diverse effects of estrogen on non-fat organs hamper the possibility of using this hormone therapeutically. To get more selective pharmacological effects of estrogens, e.g. on body fat mass, it is important to clarify their possible interactions with other fat reducing systems.

We have recently found that estrogen replacement therapy enhances the expression of leukemia inhibitory factor (LIF) receptors (9), pointing to possible interactions between the fat-suppressing effects of LIF and estrogens. LIF is a pleiotropic cytokine with extensive hematopoietic, neuronal, and endocrine actions (10), and it has also been reported that LIF can decrease fat mass in some animal models (11, 12). The LIF receptor shares the gp130 signal mediating receptor subunit with several members of the interleukin-6 (IL-6) cytokine receptor super family (10). We have recently found that endogenous IL-6 is a suppressor of body fat in mice (13, 14), and it has also been reported that ciliary neurotropic factor, another cytokine acting via the same gp130 subunit-containing receptor family, can decrease body fat mass in both rodents and humans (15–17).

In the present study, we addressed the possibility that estrogen signaling pathways are needed for the anti-obesity action of LIF. We investigated whether treatment with LIF can reduce body fat mass in ovariectomized (OVX) mice.

Materials and methods

Animals

Eight-week-old female C57Bl/6 mice were OVX or sham-operated (SHAM). After three weeks of recovery, the mice were treated with recombinant mouse leukemia inhibitory factor (LIF, 30 µg/kg; Chemicon, Temecula, CA, USA) via daily i.p. injections for 15 days ($n = 9-10$). Control ovariectomized (OVX) and sham-operated (SHAM) mice received injections of vehicle (PBS) ($n = 9-10$). Animals had free access to fresh water and standard food pellets (B&K Universal AB, Sollentuna, Sweden). All animal procedures were approved by the local ethics committee for animal care at Göteborg University and were conducted in accordance with the guidelines.

Dual X-ray absorptiometry

We have previously developed and evaluated a combined dual X-ray absorptiometry (DXA) image analysis procedure for *in vivo* imaging of mice (18). The DXA measurements were carried out with the Norland pDEXA Sabre (Norland Medical System, Fort Atkinson, WI, USA) and the Sabre research software (3.9.2) (Sabre, London, UK). Three animals were analyzed in each scan. A mouse, which was killed at the beginning of the experiment, was included in all the scans as an internal standard in order to avoid interscan variations. The crown-rump length was measured using the ruler tool, and it was defined as the distance between the crown of the skull and a point located in the middle of a line between the two femoral caput.

Computerized tomography

Computerized tomography (CT) was performed with the Stratec peripheral quantitative CT (pQCT) XCT Research M (v5.4B; Norland Medical Systems) operating at a resolution of 70 µm as previously described (19). Trabecular bone mineral density (BMD) was determined with a metaphyseal pQCT scan of the proximal tibia and defined as the inner 45% of the total area.

Serum levels of leptin

Serum leptin levels were measured by an ELISA kit (Crystal Chem Inc., Downers Grove, IL, USA) with intra-assay and interassay precision coefficients of variation of 5.4 and 6.9% respectively.

Statistics

Data in text, figures or tables are given as means \pm standard error of the mean (S.E.M.). *P* values of ≤ 0.05 were considered to be significant, as calculated with Student's *t*-test with Bonferroni's correction.

Results

Effect on serum leptin levels and body fat mass in LIF-treated animals

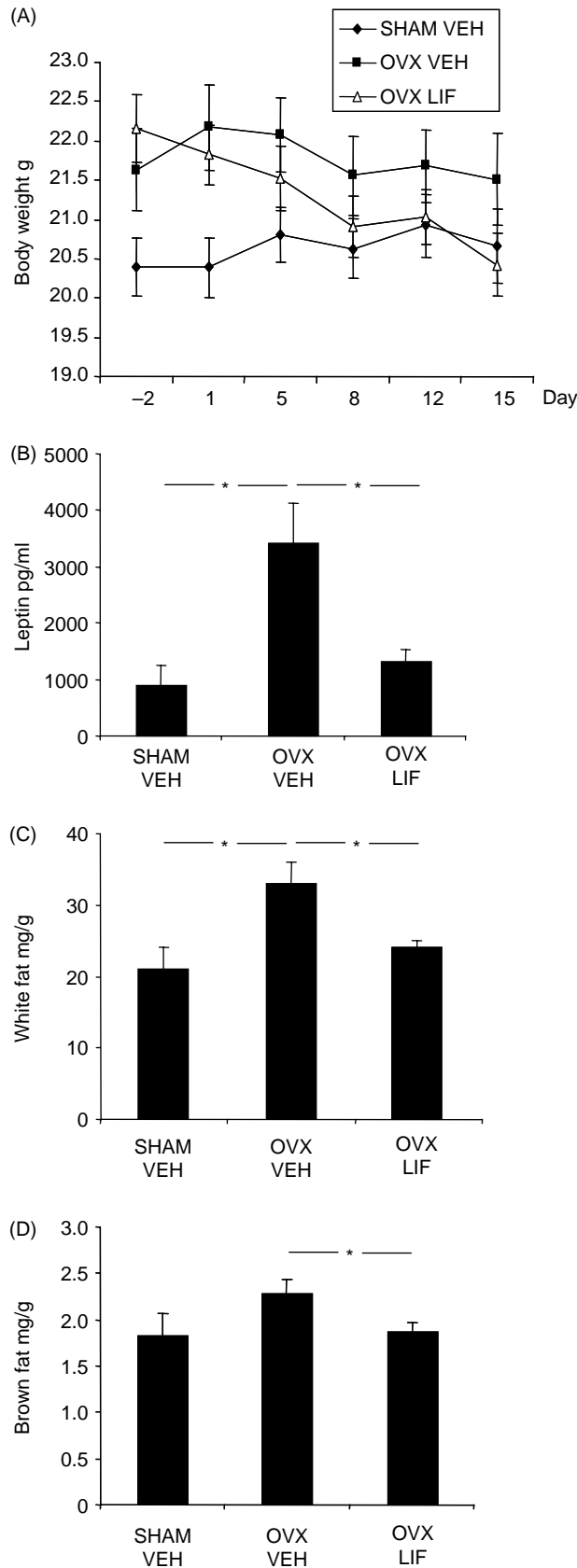
Treatment with LIF (30 µg/kg) in daily i.p. injections for 15 days to OVX mice tended to cause a decrease in body weight (8%; $P = 0.052$) compared with vehicle-treated animals (Fig. 1A). After the treatment period, the animals were killed, blood was collected, and four different fat depots were dissected: inguinal, retroperitoneal, gonadal and brown fat. The levels of the fat-derived hormone leptin in serum were increased more than threefold in OVX mice compared with SHAM mice. Treatment of OVX mice with LIF suppressed serum leptin to a level similar to that in SHAM females (Fig. 1B). The body weight of the OVX mice was 11.5 g higher after the three weeks of recovery than in sham-operated mice (Fig. 1A), and the total weight of all dissected fat pads was increased by OVX. Treatment of OVX mice with LIF suppressed total white fat weight by about 30% to a level seen in SHAM mice (Fig. 1C). The brown fat depot was decreased by 20% after treatment with LIF compared with vehicle-treated animals (Fig. 1D). The reduction in brown adipose tissue weight was not accompanied by any change in brown adipose tissue uncoupling protein (UCP)1 or UCP3 protein expression in LIF-treated mice (not shown). LIF has previously been reported to affect leptin receptor expression in white adipose tissue (20), but such an effect could not be detected in this study as the levels were not measurable even with RT-PCR.

Non-fat organ weights and bone parameters

Uterine weight was decreased by $> 80\%$ in all OVX animals and was not affected by LIF treatment (Fig. 2A). The decrease in uterine weight was also used as a positive control for a successfully performed operation. OVX mice had increased thymus weight and LIF treatment reversed this effect (Fig. 2B). The relative weights of the heart, liver and kidneys were not affected by OVX or by LIF treatment (Table 1). LIF treatment did not have any effect on longitudinal growth since the crown-rump length and the femur length were not changed. Treatment with LIF did not prevent the OVX-induced reduction in trabecular BMD (Table 1).

Discussion

It is well recognized that endogenous estrogens decrease body fat, especially visceral fat, in both humans and rodents (5–7). In line with this, we observed a significant increase in both the total weight of dissected white fat depots and in serum leptin levels after ovariectomy in the present study. Moreover, treatment with the cytokine, LIF, can decrease body fat in various animal models (11, 12).



It has been shown that estradiol replacement therapy to OVX mice enhances the expression of LIF receptors (9) and, in some but not all studies, the expression of LIF itself (10). Therefore, LIF and estradiol may interact. However, the results of the present study demonstrate that treatment with LIF can decrease body white fat mass in OVX mice in the absence of signaling by ovarian estrogens. LIF also decreased the weight of brown adipose tissue, possibly reflecting a decrease in fat storage in this tissue (21, 22). Taken together, these findings indicate that LIF-induced suppression of body fat in mice is not dependent on endogenous ovary-derived estrogens.

The decrease in brown fat mass was not accompanied by an effect on UCP1 protein expression, an index of thermogenesis in this tissue (23). These results do not support the hypothesis that the weight reducing effects of LIF involve increased energy expenditure through non-shivering thermogenesis. This view is supported by the work of Beretta *et al.* who found that central administration of an adeno-associated viral vector encoding LIF did not affect UCP1 protein expression (11), whereas it has been reported that LIF decreases food intake (24), suggesting that LIF induces weight loss mainly through reduced appetite. Ovariectomy, on the other hand, has been reported to increase food intake in mice (25).

A LIF-treated sham group might have been of limited value in this manuscript. The vehicle-treated OVX and SHAM mice differ substantially in baseline fat, and therefore it would have been difficult to compare the effects of LIF between these two groups. As mentioned above, LIF has exerted anti-obesity effects in various animal models (11, 12), and we can now conclude that this effect can also be seen in the absence of ovarian estrogens.

We found that LIF replacement therapy reversed the increase in thymus weight seen in OVX mice. It has been shown that LIF treatment can induce atrophy via a direct effect on the thymus (26), but this effect by LIF could also be secondary to stimulation of adrenocorticotropin and corticosterone release (27). A third possibility is that LIF suppresses thymus weight by decreasing body fat and leptin secretion (28). It is well known that ovariectomy increases thymus weight and that estrogen replacement therapy can reverse this effect (29–31). As estradiol enhances LIF receptor expression (9), it is possible that estrogen-mediated stimulation of LIF

Figure 1 Effect of treatment with vehicle (VEH) or LIF (30 µg/kg/day) in ovariectomized (OVX) or sham-operated (SHAM) mice on body weight (A), serum leptin levels (B), the sum of dissected white fat depots (C), and on brown fat (D). Values are given as pg leptin per ml serum (pg/ml) and as mg fat per gram body weight (mg/g) and are expressed as means ± S.E.M. (*n* = 9–10 mice per group). **P* < 0.05 as calculated with Student's *t*-test with Bonferroni's correction.

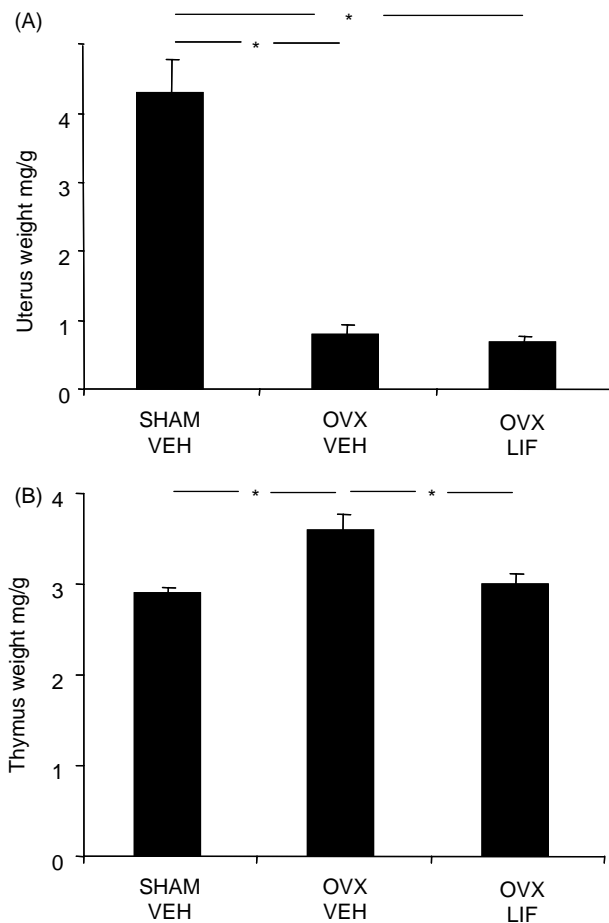


Figure 2 Weights of uterus (A) and thymus (B) after treatment with vehicle (VEH) or LIF (30 μ g/kg body weight/day in daily i.p. injections for 15 days) in ovariectomized (OVX) or sham-operated (SHAM) mice. Values are given as weight in mg per gram body weight (mg/g) and are expressed as means \pm S.E.M. ($n = 9-10$). * $P < 0.05$ as calculated with Student's t -test with Bonferroni's correction.

Table 1 Non fat organ weights and bone parameters after treatment with vehicle (VEH) or LIF (30 μ g/kg body weight/day in daily i.p. injections for 15 days) in ovariectomized (OVX) or sham-operated (SHAM) mice. Values are given as organ weight in milligram per gram body weight (mg/g), length in mm, and BMD in mg/cm³ and expressed as mean \pm S.E.M. ($n = 9-10$).

	SHAM VEH	OVX VEH	OVX LIF
Heart (mg/g)	4.90 \pm 0.18	5.13 \pm 0.15	5.11 \pm 0.06
Kidney (mg/g)	12.30 \pm 0.23	12.05 \pm 0.29	12.3 \pm 0.22
Liver (mg/g)	45.10 \pm 0.72	40.93 \pm 1.51	44.53 \pm 1.64
Crown-rump length (mm)	70.96 \pm 0.45	70.54 \pm 1.04	70.00 \pm 0.85
Femur length (mm)	ND	15.8 \pm 0.14	16.16 \pm 0.11
Trabecular BMD (mg/cm ³)	288.6 \pm 9.1	218.7 \pm 6.81	214.7 \pm 4.61 \dagger

Crown-rump length and femur length were measured by DXA while pQCT analyses of the metaphyseal region of distal tibia were used for measuring trabecular bone mineral density (BMD).

ND, not determined, $\dagger P < 0.05$ vs SHAM VEH as calculated with Student's t -test with Bonferroni's correction.

responsiveness also mediates this effect of endogenous estrogens. In any event, the thymus reducing effect of LIF does not seem to be dependent on endogenous estrogens.

LIF treatment did not reverse the marked decrease in uterine weight or trabecular BMD in ovariectomized mice. These could be examples of LIF-independent biological effects by endogenous estrogens. The absence of a LIF effect could be due to a tissue-specific marked lack of LIF receptors in bone and uteri of OVX mice. The lack of LIF receptors could, in turn, be caused by a more pronounced dependence on estrogens in these organs (9).

The suppression of fat by endogenous estrogens has been shown to be mediated by estrogen receptor (ER) alpha (7, 32). Estrogens have been shown to increase LIF receptor expression via ER alpha (9). We here show that LIF decreases obesity in OVX mice. Based on these results in conjunction, it can be speculated that the ER alpha-mediated fat suppressing effect of estrogens is caused by enhanced LIF efficacy. However, more studies are needed to evaluate this possibility. There are few studies investigating the possibility that LIF affects estrogen secretion, although it has been reported that transfection with LIF-expressing plasmids locally in the pituitary suppresses gonadotropin secretion (33) and presumably also estrogen secretion. It seems unlikely that LIF suppressed the very low estrogen levels in OVX mice, not least because decreased estrogen secretion would increase, rather than decrease, fat mass and thymus weight.

The results of the present study, that LIF suppresses fat mass in OVX mice, are in line with previous results that systemic LIF treatment to monkeys (12) and transfection with LIF-expressing virus particles to the brain of rats (11) decreases body fat. This may be part of a generalized effect of cytokines that activate the IL-6 receptor subunit, gp130. We have shown that endogenous IL-6 itself selectively decreases fat mass (13, 14) and other groups have demonstrated that the IL-6-related cytokine ciliary neurotropic factor decreases body fat mass without affecting lean body mass (15, 17). Interestingly, the latter cytokine also exerted long-term fat suppressing effects in early clinical studies (16). Thus, there is growing evidence in the literature that several members of the cytokine family, acting via gp130, can decrease body fat mass in healthy individuals without clinical inflammation. Moreover, several of the gp130-regulating cytokines may exert their effect via the brain (11, 13, 14, 17, 34, 35).

In conclusion, we have shown that peripheral LIF treatment decreases body fat mass in OVX mice. This indicates that the fat-reducing effect of LIF does not require signaling by estrogens, another group of fat-suppressing hormones that previously have been reported to interact with the LIF system.

Acknowledgements

We thank the Swedish Research Council (52120035116, 52120034312), the Novo Nordisk Foundation, the Swedish Medical Society, the Swedish Society for Medical Research and the Swedish Strategic Foundation. We acknowledge the Center for BioImaging, Göteborg University for technical assistance.

This research was supported by EC FP6 funding (contract no. LSHMCT 2003503041). This publication reflects the author's views and not necessarily those of the EC. The information in this document is provided as is and no guarantee or warranty is given that the information is fit for any particular purpose. The user thereof uses the information at their sole risk and liability.

References

- Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 2004 **116** 337–350.
- Dohrmann CE. Target discovery in metabolic disease. *Drug Discovery Today* 2004 **9** 785–794.
- Robinson SW, Dinulescu DM & Cone RD. Genetic models of obesity and energy balance in the mouse. *Annual Reviews in Genetics* 2000 **34** 687–745.
- Reitman ML. Metabolic lessons from genetically lean mice. *Annual Reviews in Nutrition* 2002 **22** 459–482.
- Richter CP & Uhlenhuth EH. Comparison of the effects of gonadectomy on spontaneous activity of wild and domesticated Norway rats. *Endocrinology* 1954 **54** 311–322.
- Mook DG, Kenney NJ, Roberts S, Nussbaum AI & Rodier WI 3rd. Ovarian-adrenal interactions in regulation of body weight by female rats. *Journal of Comprehensive Physiology and Psychology* 1972 **81** 198–211.
- Ohlsson C, Hellberg N, Parini P, Vidal O, Bohlooly M, Rudling M, Lindberg MK, Warner M, Angelin B & Gustafsson JA. Obesity and disturbed lipoprotein profile in estrogen receptor- α -deficient male mice. *Biochemistry and Biophysics Research Communications* 2000 **278** 640–645.
- Tchernof A, CallesEscandon J, Sites CK & Poehlman ET. Menopause, central body fatness, and insulin resistance: effects of hormone replacement therapy. *Coronary Artery Disease* 1998 **9** 503–511.
- Lindberg MK, Moverare S, Eriksson AL, Skrtic S, Gao H, DahlmanWright K, Gustafsson JA & Ohlsson C. Identification of estrogen-regulated genes of potential importance for the regulation of trabecular bone mineral density. *Journal of Bone Mineral Research* 2002 **17** 2183–2195.
- Auernhammer CJ & Melmed S. Leukemia inhibitory factor neuroimmune modulator of endocrine function. *Endocrine Reviews* 2000 **21** 313–345.
- Beretta E, Dhillon H, Kalra PS & Kalra SP. Central LIF gene therapy suppresses food intake, body weight, serum leptin and insulin for extended periods. *Peptides* 2002 **23** 975–984.
- Akiyama Y, Kajimura N, Matsuzaki J, Kikuchi Y, Imai N, Tanigawa M & Yamaguchi K. *In vivo* effect of recombinant human leukemia inhibitory factor in primates. *Japanese Journal of Cancer Research* 1997 **88** 578–583.
- Wallenius K, Wallenius V, Sunter D, Dickson SL & Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochemistry and Biophysics Research Communication* 2002 **293** 560–565.
- Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C & Jansson JO. Interleukin-6-deficient mice develop mature-onset obesity. *Nature Medicine* 2002 **8** 75–79.
- Gloaguen I, Costa P, Demartis A, Lazzaro D, Di Marco A, Graziani R, Paonessa G, Chen F, Rosenblum CI, Van der Ploeg LH, Cortese R, Ciliberto G & Laufer M. Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. *PNAS* 1997 **94** 6456–6461.
- Ettinger MP, Littlejohn TW, Schwartz SL, Weiss SR, McIlwain HH, Heymsfield SB, Bray GA, Roberts WG, Heyman ER, Stambler N, Heshka S, Vicary C & Guler HP. Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. *Journal of the American Medical Association* 2003 **289** 1826–1832.
- Lambert PD, Anderson KD, Sleeman MW, Wong V, Tan J, Hijarunguru A, Corcoran TL, Murray JD, Thabet KE, Yancopoulos GD & Wiegand SJ. Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat without cachexia or rebound weight gain, even in leptin-resistant obesity. *PNAS* 2001 **98** 4652–4657.
- Sjogren K, Hellberg N, Bohlooly YM, Savendahl L, Johansson MS, Berglindh T, Bosaeus I & Ohlsson C. Body fat content can be predicted in vivo in mice using a modified dual-energy X-ray absorptiometry technique. *Journal of Nutrition* 2001 **131** 2963–2966.
- Windahl SH, Vidal O, Andersson G, Gustafsson JA & Ohlsson C. Increased cortical bone mineral content but unchanged trabecular bone mineral density in female ERbeta(-/-) mice. *Journal of Clinical Investigations* 1999 **104** 895–901.
- Meli R, Pacilio M, Raso GM, Esposito E, Coppola A, Nasti A, Di Carlo C, Nappi C & Di Carlo R. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. *Endocrinology* 2004 **145** 3115–3121.
- Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK & Lowell BB. BetaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 2002 **297** 843–845.
- Yu S, Gavrilova O, Chen H, Lee R, Liu J, Pacak K, Parlow AF, Quon MJ, Reitman ML & Weinstein LS. Paternal versus maternal transmission of a stimulatory G-protein alpha subunit knockout produces opposite effects on energy metabolism. *Journal of Clinical Investigations* 2000 **105** 615–623.
- Lowell BB & Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature* 2000 **404** 652–660.
- Plata-Salaman CR. Cytokines and feeding suppression: an integrative view from neurologic to molecular levels. *Nutrition* 1995 **11** 674–677.
- Pallier E, Aubert R & Lemonnier D. Effect of diet and ovariectomy on adipose tissue cellularity in mice. *Reproduction, Nutrition and Development* 1980 **20** 631–636.
- Mito N, Yoshino H, Hosoda T & Sato K. Analysis of the effect of leptin on immune function in vivo using diet-induced obese mice. *Journal of Endocrinology* 2004 **180** 167–173.
- Sempowski GD, Hale LP, Sundry JS, Massey JM, Koup RA, Douek DC, Patel DD & Haynes BF. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and are associated with thymic atrophy. *Journal of Immunology* 2000 **164** 2180–2187.
- Howard JK, Lord GM, Matarese G, Vendetti S, Ghattei MA, Ritter MA, Lechler RI & Bloom SR. Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. *Journal of Clinical Investigation* 1999 **104** 1051–1059.
- Erlandsson MC, Ohlsson C, Gustafsson JA & Carlsten H. Role of oestrogen receptors alpha and beta in immune organ development and in oestrogen-mediated effects on thymus. *Immunology* 2001 **103** 17–25.
- Marotti T, Sirotkovic M, Pavelic J, Gabrilovac J & Pavelic K. *In vivo* effect of progesterone and estrogen on thymus mass and T-cell functions in female mice. *Hormone and Metabolic Research* 1984 **16** 201–203.
- Oner H & Ozan E. Effects of gonadal hormones on thymus gland after bilateral ovariectomy and orchidectomy in rats. *Archives in Andrology* 2002 **48** 115–126.

- 32 Heine PA, Taylor JA, Iwamoto GA, Lubahn DB & Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *PNAS* 2000 **97** 12729–12734.
- 33 Yano H, Readhead C, Nakashima M, Ren SG & Melmed S. Pituitary-directed leukemia inhibitory factor transgene causes Cushing's syndrome: neuro-immune-endocrine modulation of pituitary development. *Molecular Endocrinology* 1998 **12** 1708–1720.
- 34 Li G, Klein RL, Matheny M, King MA, Meyer EM & Scarpace PJ. Induction of uncoupling protein 1 by central interleukin-6 gene delivery is dependent on sympathetic innervation of brown adipose tissue and underlies one mechanism of body weight reduction in rats. *Neuroscience* 2002 **115** 879–889.
- 35 Stenlof K, Wernstedt I, Fjallman T, Wallenius V, Wallenius K & Jansson JO. Interleukin-6 levels in the central nervous system are negatively correlated with fat mass in overweight/obese subjects. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 4379–4383.
-

Received 9 June 2005

Accepted 28 October 2005