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The Decrease of Serum Leptin Levels in Oestrogen-Treated Male Mice

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Summary

Adipocyte hormone leptin (OB protein) is considered to be an "adiposity signal" regulating body weight homeostasis and energy balance. We have previously reported that oestrogens (oestradiol-benzoate) significantly decrease the body weight in male rats, increase anterior pituitary and serum levels of the intracellular messenger cAMP, which activates cAMP-dependent protein kinase A , their targets include hormone-sensitive lipase and they influence the brain sympathetic system. The present study tested our hypothesis that oestrogens could influence serum leptin levels in male mice. We found that chronic administration of oestradiol-benzoate significantly attenuated serum levels of leptin, in the dependence on the duration of its administration, and simultaneously decreased body weight. We suppose that oestrogens affect leptin levels interacting with the signal transmission system of cAMP, possibly at the genome level. Our observations that the food consumption of mice with simultaneously decreased body weight and levels of serum leptin support the idea that there exists a satiety factor that counters the effect of low leptin.

Key words
Oestrogens – Oestradiol-benzoate – Leptin – cAMP – Body weight – Mice

Introduction

The *ob* gene and its protein product were identified by Zhang *et al.* (1994). The *ob* protein, termed "leptin" from the Greek leptos, meaning thin, is produced in adipose tissue and is thought to act as an afferent satiety signal in a feedback loop that putatively affects the appetite and satiety centres of the brain (Rohner-Jeanrenaud and Jeanrenaud 1996). It was recently observed that the leptin secretion and blood levels are increased in obesity and following fat consumption (Frederich *et al.* 1995, Considine *et al.* 1996) and that the administration of leptin to *ob/ob* mice causes weight loss by influencing energy intake and by increasing their energy expenditure (Campfield *et al.* 1995, Halaas *et al.* 1995, Hebebrand *et al.* 1995) and these animals become fertile (Chehab *et al.* 1996). Zhang *et al.* (1994) predicted that this hormone has the hypothalamus as its target. The research teams from Millenium Pharmaceutical (Cambridge, Massachusets) and Roche Research Gent (a division of Hoffman-La Roche in Gent, Belgium) reported that they may have found molecules that mediate the effects of leptin in the body, namely two related receptors for leptin from humans and mice (Barinaga 1996). Collins (Collins *et al.* 1996, Collins and Surwit 1996) recently showed that leptin seems to be a sensor of adipocyte mass, and that treatment with a thermogenic beta-3-adrenergic receptor agonist reduces both adipose tissue mass and leptin expression (Collins *et al.* 1996). Therefore, they hypothesized that leptin's role in regulating fat mass might include signalling for the sympathetic nervous system to increase thermogenesis and energy expenditure in brown adipose tissue with increasing fat mass.

Our previous study (Nedvídková *et al.* 1992, Schreiber *et al.* 1993) showed that oestrogens significantly reduced body weight, affected the central adrenergic nervous system, and that they especially attenuated the levels of norepinephrine, epinephrine

and dopamine in the anterior pituitary of male rats (Nedvídková *et al.* 1996b. Based on these results and the proposition that leptin acts in the brain to lower food intake and adiposity, we measured immunoreactive leptin levels in the serum of male mice treated with oestradiol benzoate.

Material and Methods

Normal male mice (initial body weight 22–25 g) were purchased from Velaz Farm (Prague, Czech Republic) and housed at room temperature 24±2 °C under a 12-h light/dark cycle with food and water *ad libitum*, the animals were randomly divided into four experimental groups (one control, three OB-groups), with 20 mice in each group. Mice received a microcrystaline suspension of oestradiol-benzoate (OB, Agofollin-Depot, Biotika) in a dose of 10 mg/kg i.m. twice a week, or a vehicle (0.9 % saline) i.m. The experiment was terminated after one, two or three weeks, the mice were weighed, killed by decapitation in the morning, blood was withdrawn, centrifuged, and the serum was stored at −20 °C until analyzed. The mouse leptin was determined by a radioimmunoassay kit (WAK-Chemie Medical GMBH). This mouse leptin assay has been developed to measure mouse leptin in the plasma or serum. It is a completely homologous assay since the antibody was raised against highly purified mouse leptin and both the standard and tracer are prepared with mouse leptin. The limit sensitivity for the mouse leptin assay is 0.2 ng/ml, the limit of linearity is 20 ng/ml. The intraassay variability was 6.7±1.5 %, interassay variability 10.8±2.0 %.

Statistical analysis was carried out using the Student's t-test for paired and unpaired data.

Table 1. Serum levels of leptin after one week, two or three weeks lasting treatment with oestradiol-benzoate (OB), and the % body weight changes after OB.

Animal groups (duration of OB administration)	The increase of body weight in %	Serum leptin (ng/ml)
Controls (3 weeks)	71±3.0	4.2±1.1
1 week OB	−1.3±0.2**	2.2±0.6*
2 weeks OB	7.7±0.9**	2.0±0.9**
3 weeks OB	25.2±1.8**	1.6±0.4**

*The values are means ± S.D., n=number of animals in each group. *=P<0.05 vs. control group, **=P<0.001 vs. control group*

Results and Discussion

Contrary to our expectations, the serum levels of leptin were significantly decreased proportionally to the duration of oestradiol-benzoate (OB) administration (Table 1). Concomitantly, OB lowered the body weight of mice. Thus, the leptin levels in this experiment just reflected the body mass.

We found that twice weekly i.m. injection of oestradiol benzoate reduced the body weight and serum levels of leptin in dependence on the duration of OB administration, while an essentially normal food intake was maintained.

Jeffrey Friedman's team at Rockfeller University cloned the obese gene *ob* which, when mutated, caused mice to become grossly fat. These authors showed that its protein product, leptin, is a key weight-controlling hormone (Zhang *et al.* 1994).

Leptin exerts its effect through recently encoded receptors, which are present within various peripheral tissues and, in considerably smaller amounts, in the hypothalamus and chorioid plexus (Chehab *et al.* 1996). Many scientists believe that leptin is signalling factor for body weight homeostasis which reduces food intake by direct binding to receptors in the central nervous system (Tartaglia *et al.* 1995). Its secretion and blood levels are increased in obesity (Considine *et al.* 1996) and following fat consumption (Frederich *et al.* 1995). On the other hand, fasting decreased serum leptin levels (Saladin *et al.* 1995). Our data demonstrate that the administration of oestradiol-benzoate unexpectedly and significantly decreased serum levels of leptin and the body weight of mice, e.g., like starving or food intake reduction. On the other hand, increased body weight is frequently observed in women after oestrogen treatment. Saladin *et al.* (1995) showed that the *ob* gene exhibits diurnal variation, increasing during the night after the rats start to eat. This variation was linked to changes in food intake, since fasting prevented the cyclic variation and decreased *ob* messenger RNA, refeeding of fasted rats restored *ob* mRNA within 4 hours to levels of fed controls. Rentsch *et al.* (1995) observed that, after a single intravenous injection, the *ob* gene product decreased food intake after fasting in normal mice. It is known that brown fat is an important site of adrenaline- and noradrenaline-stimulated energy expenditure (Himms-Hagen 1989). Noradrenaline released from sympathetic nerve endings stimulates two different types of receptors at the cell surface, alpha- and beta-adrenoceptors, the latter causing increased cAMP and activation of cAMP-dependent protein kinase A (PKA). In a previous study, we showed that oestradiol-benzoate stimulated serum and anterior pituitary levels of intracellular messenger cAMP (Nedvídková *et al.* 1992, Schreiber *et al.* 1993), and changed the anterior pituitary contents of catecholamines and its receptors (Nedvídková *et al.*

1996a,b). It may be assumed that higher cAMP levels after oestradiol-benzoate can enhance PKA activation. PKA is a tetrameric protein composed of two monomeric catalytic subunits (C) and a dimeric regulatory (R) subunit (RI and RII isoforms) that prevents the enzyme from acting when cAMP is not beound (Cummings *et al.* 1996). RII, but not RI, isoforms, are phosphorylated in the autoinhibitory domain by C, reducing the affinity of R for C. In RIIβ-knockout mice (Cummings *et al.* 1996), chronic PKA "overactivity" in brown fat stimulated whole-body energy expenditure, and mice lost fat while essentially normal food intake was maintained. This leanness in mutant mice was associated with a marked reduction in the levels of messenger RNA and circulating leptin, the adipocyte-secreted satiety factor absent in *ob/ob* mice (Zhang *et al.* 1994). A similar effect was observed in our experiments reported here. We therefore suggest that oestrogens could operate at the level of genome expression of the PKA RII subunit. As leptin deficiency in ob/ob mice causes increased food intake, it is unexpected that RIIβ-deficient mice, and our oestrogenized mice with low leptin levels, do not exhibit compensatory hyperphagia. It is possible that there is a satiety factor present that counters the effects of low leptin levels. Stephens *et al.* (1995) observed that neuropeptide Y stimulates food intake, decreases thermogenesis, and increases plasma insulin and corticosterone levels, making it a potential target. This protein also supresses prolactin secretion from rat anterior pituitary cells (Wang *et al.* 1996). One mechanism by which leptin could regulate food intake and metabolism is the inhibition neuropeptide Y synthesis and release. This peptide also counters oestrogen effect on adrenergic pathways. Collins *et al.* (1996) further demonstrated that treatment with a thermogenic beta-3-adrenergic receptor agonist reduced both adipose tissue mass and leptin expression. Nevertheless, more detailed investigation of oestrogens, catecholamines, neuropeptides and leptin regulative interactions is necessary for understanding the factors regulating body weight homeostasis.

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Reprint requests

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