

Does leptin modulate immune and endocrine response in the time of LPS-induced acute inflammation?

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Abstract

OBJECTIVES: In many studies it has been reported, that leptin may play an important role not only in the regulation of food intake and body weight but can modify immune response. The aim of our study was to estimate the effects of the administration of exogenous leptin on serum concentration of proinflammatory cytokines (interleukin 6-IL 6 and tumor necrosis factor alpha-TNF α) and anti-inflammatory cytokine (interleukin 10 - IL 10) during LPS induced acute inflammation. We also estimated leptin's influence on pituitary, thyroid, adrenal and gonadal hormones in response to lipopolisaccharyde (LPS) induced acute inflammation.

METHODS: Male rats Wistar-Kyoto were divided into four groups, which received respectively: placebo (0.9% NaCl), LPS, leptin and leptin with LPS. The TNF α and IL 6 serum concentrations were measured after 2 hours and IL 10 after 4 hours. The pituitary, thyroid, adrenal and gonadal hormones serum concentrations were measured after 2 and 4 hours. Cytokine concentrations were estimated using ELISA tests and hormones concentrations using RIA tests.

RESULTS: Leptin did not have an effect on both cytokine responses (pro-inflammatory and anti-inflammatory) in the time of LPS-induced acute inflammation. Leptin enhanced LPS-induced increasing of corticosterone secretion after 2 hours and decreased LPS-induced inhibition of testosterone secretion after 4 hours.

CONCLUSIONS: Leptin can modulate hormone response during LPS-induced acute inflammation.

Introduction

Leptin – product of OB gene, is peptide adipocyte derived hormone. Leptin and neuropeptide Y (NPY) are known to keep a key position in the regulation of food intake and energy balance [1–3]. There is some information that synthetic leptin agonists may be useful in the treatment of human obesity [4]. Research of leptin is developing very fast and indicates, that the role of this hormone is not only limited to control of energy homeostasis. Leptin exerts its actions through its receptor (OB-R), which has high affinity to gp 130 receptor, structurally related to 1st class cytokine receptors. Leptin's receptor was found in different organs such as: kidneys, liver, heart, lungs, pituitary gland, testes, ovaries, uterus, adipose tissues and haematopoietic tissues [5–9]. The fact that leptin's receptor appears in many organs indicates, that this hormone may play various roles in the whole mammalian organism. Leptin can modulate apoptosis because central administration of this hormone in rats results in losing adipocytes by increasing apoptosis [10] and also can modulate hematopoiesis and function of macrophages [9]. It was shown in experimental studies that leptin administration in ob/ob mice decreased LPS-induced lethality and sensitivity for LPS [11, 12]. Exogenous leptin regulates phagocytic function of macrophages in ob/ob mice [13]. This hormone stimulates IL 2 and INF γ productions by Th1 lymphocytes [14–15]. The endocrine system also takes part in response against acute inflammation by mobilizing hypothalamus-pituitary-adrenal axis and decreasing activity pituitary-thyroid axis [16–19]. Leptin's influence on the endocrine system is still unclear [5, 20]. The aim of our study was to estimate the effects of exogenous leptin administration on the serum concentrations of proinflammatory cytokines (IL 6 and TNF α) and anti-inflammatory cytokine (IL10) and we also estimated leptin's influence on pituitary, thyroid, adrenal and gonadal hormones in response to LPS induced acute inflammation.

Material and methods

For all experiments male Wistar-Kyoto rats (250–300 g) were used. The animals were kept under controlled light (LD 10 : 14 h) and temperature (22^o C) with free access to pelleted food and water ad libitum. The animals were divided into following groups which received intraperitoneally respectively:

- Group 1: 14 animals received 150 μ l 0.9% NaCl.
- Group 2: 17 animals received 600 μ g LPS (*Esh-erichia coli*; 055: BT Sigma).
- Group 3: 15 animals received 300 μ g murine leptin. (PeproTech; England).

- Group 4: 19 animals received 600 μ g LPS (*Esh-erichia coli*; 055: BT Sigma) and 300 μ g murine leptin. (PeproTech; England).

Then the animals were killed by decapitation after 2 or 4 hours. Trunk blood was collected and the serum separated and stored at –20°C.

All experimental procedures and protocols were in accordance with Guidelines for the Care and Use of Experimental Animals (Endocrine Society).

The following cytokines were measured using ELISA tests after 2 hours: IL 6 (Endogen, USA) and TNF α (Amersham Pharmacia Biotech, England).

The following cytokine was measured using ELISA test after 4 hours: IL 10 (Endogen, USA)

The following hormones were evaluated by RIA assays after 2 and 4 hours: rat serum LH and PRL were measured by RIA kits provided by Dr. A.F. Parlow and NIDDK (USA), T3, T4, testosterone using kits (Orion Diagnostica, Finland), corticosterone using kit (ICN Biomedicals Inc, USA)

Data were analysed by unpaired Student test and analyses of variance applied to determine significant differences between groups. Results are expressed as the mean \pm SEM and the statistical significance is accepted at $p < 0.05$.

Results

LPS administration caused increasing TNF α and IL 6 serum concentrations after 2 hours and IL 10 after 4 hours in comparison with the control group ($p < 0.01$, $p < 0.01$, $p < 0.01$ respectively Table 1).

LPS decreased serum concentrations of T4 after 2 hours ($p < 0.05$ Table 2) and T3, T4, testosterone and prolactin after 4 hours ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$ respectively Table 3), however serum corticosterone levels were increased in response to LPS after 2 hrs ($p < 0.05$) and after 4 hrs ($p < 0.05$).

Leptin administration demonstrated decreasing of corticosterone after 2 hours compared to the control group ($p < 0.05$ Table 4).

The following results were recorded by comparison group received LPS and leptin with group received only LPS:

1. Decreasing T3 serum concentration after 2 hours ($p < 0.05$ Figure 1)
2. Decreasing LH serum concentration after 2 hours ($p < 0.05$ Figure 2)
3. Increasing testosterone serum concentration after 4 hours ($p < 0.01$ Figure 3)
4. Increasing corticosterone serum concentration after 2 hours ($p < 0.01$ Figure 4)
5. There is no differences in serum cytokine concentrations between group received LPS plus leptin compared to group receiving only LPS.

Table 1. Mean (\pm SEM) serum cytokine concentrations after LPS administration at 2 and 4 hours in comparison with control group.

	LPS	0.9 % NaCl	p
IL 6 (pg/ml) after 2 hours	26207 \pm 3686 (n = 7)	51.4 \pm 1.87 (n = 7)	< 0.01
TNF alfa (pg /ml) after 2 hours	1427 \pm 255 (n = 7)	11.5 \pm 1.57 (n = 7)	< 0.01
IL 10 (pg/ml) after 4 hours	2068 \pm 198 (n = 10)	56.87 \pm 9.74 (n = 7)	< 0.01

Table 2. Mean (\pm SEM) serum hormones concentrations after LPS administration at 2 hours in comparison with control group.

	LPS	0.9 % NaCl	p
IL 6 (pg/ml) after 2 hours	26207 \pm 3686 (n = 7)	51.4 \pm 1.87 (n = 7)	< 0.01
TNF alfa (pg /ml) after 2 hours	1427 \pm 255 (n = 7)	11.5 \pm 1.57 (n = 7)	< 0.01
IL 10 (pg/ml) after 4 hours	2068 \pm 198 (n = 10)	56.87 \pm 9.74 (n = 7)	< 0.01

Table 3. Mean (\pm SEM) serum hormones concentrations after LPS administration at 4 hours in comparison with control group.

	LPS	0.9 % NaCl	p
T3 (nmol/l)	1.01 \pm 0.05 (n = 10)	1.42 \pm 0.05 (n= 7)	< 0.01
T4 (nmol/l)	37.6 \pm 1.8 (n = 10)	50.39 \pm 4 (n = 7)	< 0.01
Testosterone (nmol/l)	0.13 \pm 0.01 (n = 10)	2.23 \pm 0.75 (n=7)	< 0.05
Corticosterone (nmol/l)	1245 \pm 108 (n =10)	617.3 \pm 202 (n = 7)	< 0.05
Prolactin (μ g/l)	1.89 \pm 0.16 (n = 10)	4.92 \pm 1.15 (n = 7)	< 0.05

Table 4. Mean (\pm SEM) serum hormones concentrations after leptin administration at 2 hours in comparison with control group.

	leptin	0.9 % NaCl	p
Corticosterone (ng/ml)	313 \pm 105.6 (n = 7)	728 \pm 175 (n = 7)	< 0.05

Discussion

Present studies show that the role of leptin is not only limited to controlling body weight and energetic balance. Leptin's receptors are represented in many organs and this fact shows that this hormone can regulate the functions of the whole body including the endocrine and immune systems. The effect of leptin on the endocrine system is still unclear. The existence of negative feedback between leptin and glucocorticoids secretion is suggested. This fact is probably concerned with inverse circadian rhythms between leptin and cortisol secretion [21]. Leptin probably

inhibits hypothalamic CRH secretion [22]. On the other hand the investigation of isolated adrenal cells in rats show that leptin inhibits corticosterone secretions [23]. The correlation between thyroid hormones and leptin levels are still absolutely unclear [5,20, 24]. In our study we established that intraperitoneal leptin administration caused the decrease of serum corticosterone level after 2 hours (it can be connected with negative feedback between leptin and hypothalamus-pituitary-adrenal axis). Leptin plays an important role in regulation of immune response. It modulates productions of anti-inflammatory and proinflammatory cytokines which was confirmed

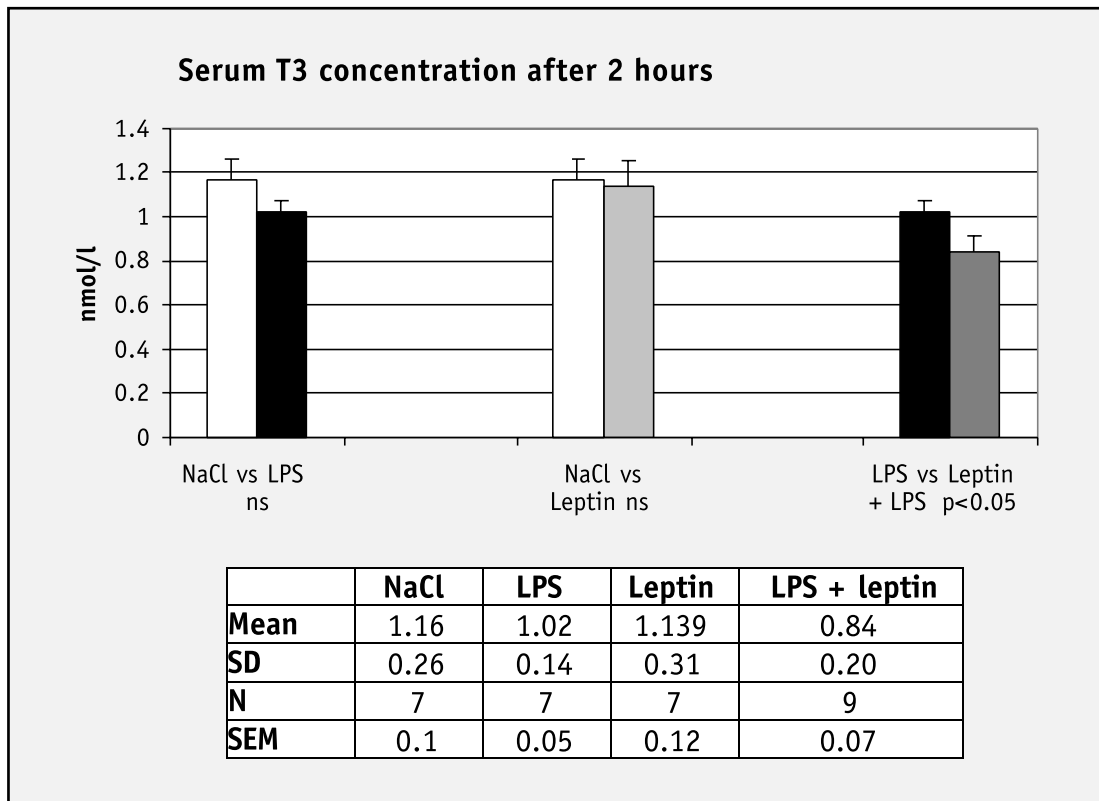


Fig. 1.

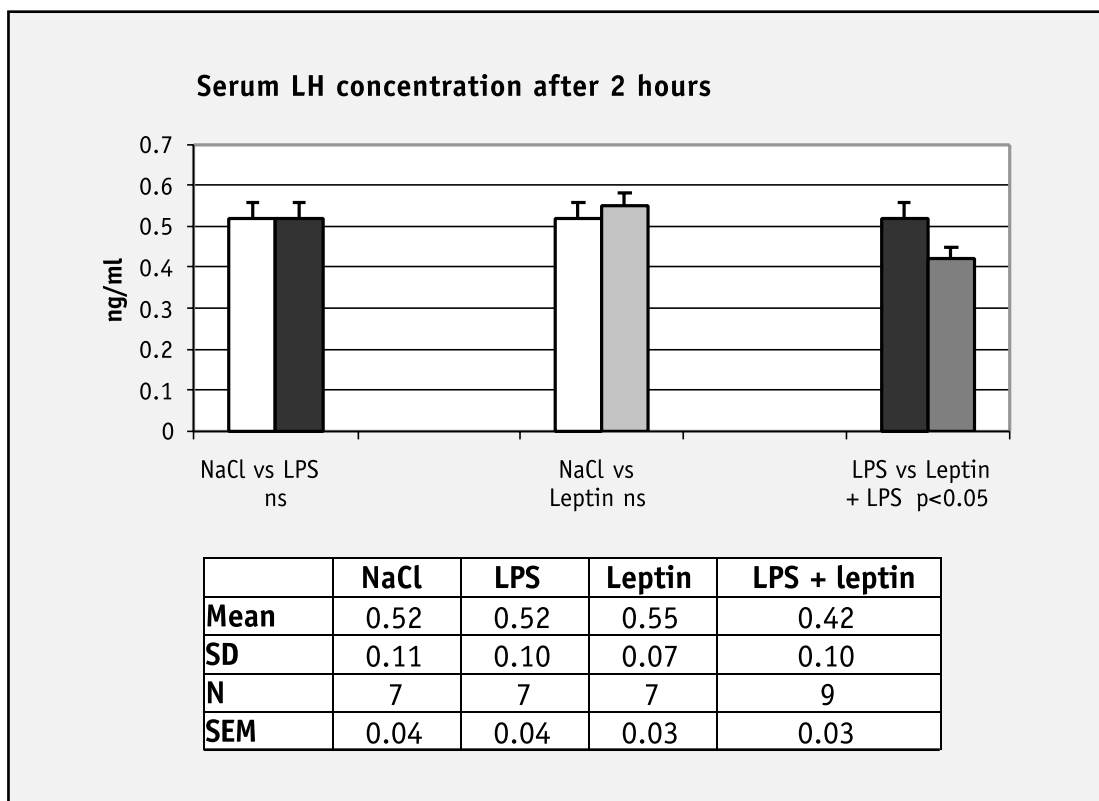


Fig. 2.

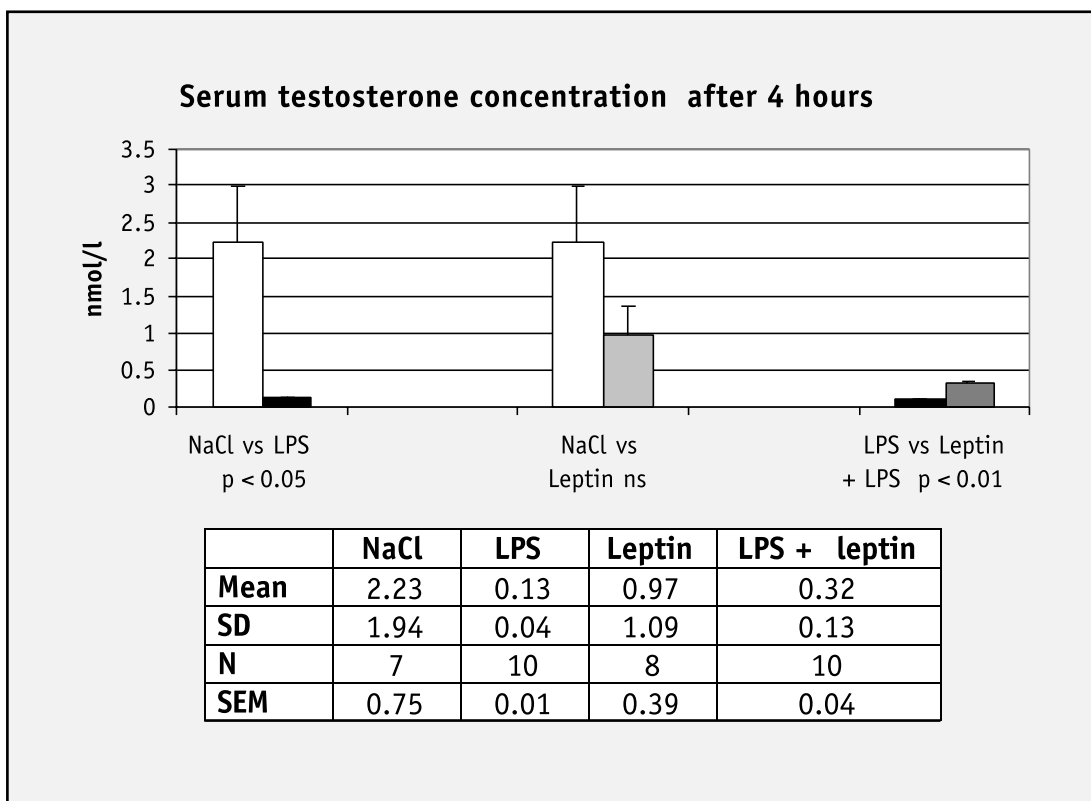


Fig. 3.

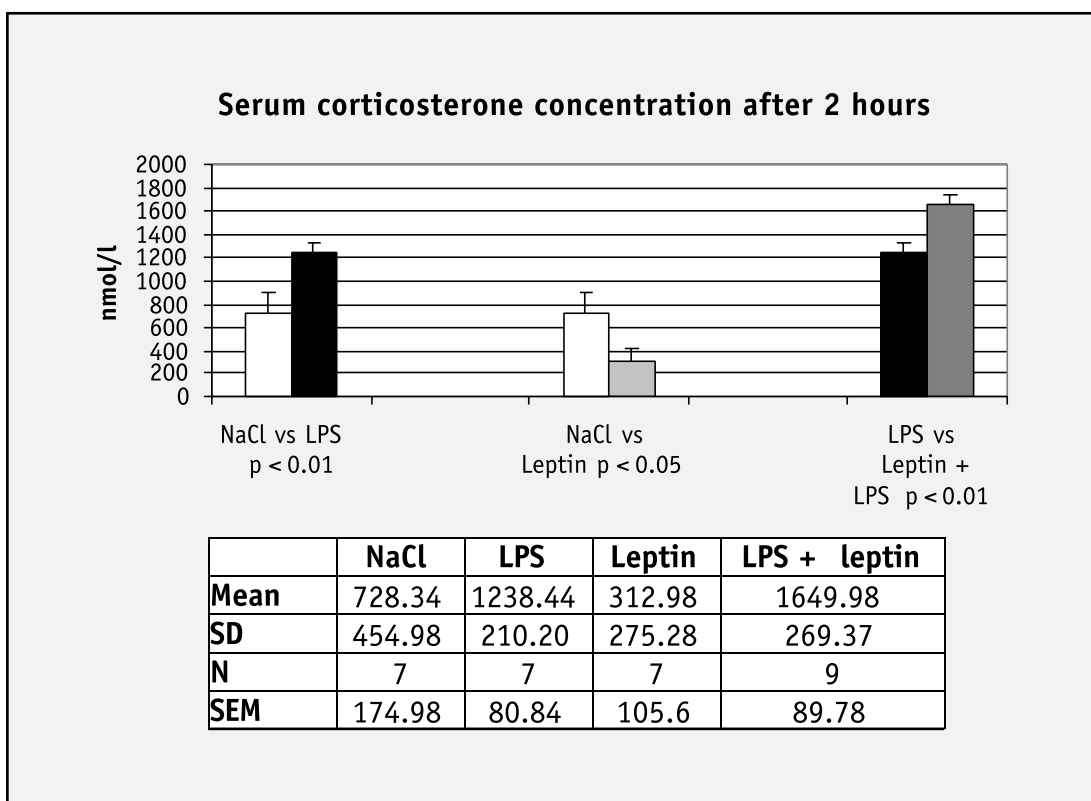


Fig. 4.

in many experimental studies followed above all on ob/ob and db/db mice [12,13]. Proinflammatory cytokines such as IL 1, TNF α or LPS administered to mice increase serum leptin levels in experimental animals [25–29]. LPS-induced lethality of experimental animals decreased after exogenous leptin administration [11–12]. Mortality of ob/ob mice induced by TNF α were lowered after leptin application [30]. Leptin improves diminished phagocytic macrophages' function in ob/ob mice [13]. In vitro studies have shown that leptin encourages activation and proliferation of monocytes [31]. It is very important because tissue macrophages and circulating monocytes have been identified as a major source of TNF α in healthy lean humans [32]. The synthesis of proinflammatory cytokines (TNF α and IL 6) in fa/fa rats is lower than in their lean littermates [13]. It was shown that leptin stimulates IL 2 and INF γ production by Th 1 lymphocytes [14,15] and inhibits IL 4 production by Th 2 lymphocytes [14]. On the other hand it was demonstrated that leptin deficiency may be a protective factor against T-cell mediated hepatotoxicity. This fact can be dependent upon of lower synthesis TNF α and IL 18 which is connected with lack of leptin [33]. Our study indicates that leptin did not modulate serum cytokine levels (proinflammatory TNF α and IL 6 and anti-inflammatory IL 10) in response to LPS induced acute inflammation. It is important to say that the majority of studies were followed on leptin deficient or receptor deficient animals but not on healthy animals.

Response to acute inflammation is regulated by the immune and endocrine systems. The reaction of the endocrine system is expressed by the mobilization of the hypothalamic-pituitary-adrenal axis and the inhibition of pituitary-thyroid axis [16–19]. Our study showed that leptin enhanced LPS induced increase of corticosterone secretion. It was also found that T3 secretion decrease correlated with LPS application was amplified by the combination LPS and leptin. The results demonstrate that leptin reduced the inhibiting role of LPS in testosterone secretion. These results confirmed leptin's influence on endocrine reply to LPS-induced acute inflammation. Bacterial infections increase productions of many cytokines (such as IL 1, IL 2, IL 6, TNF α) which directly and indirectly stimulate adrenal-corticosterone secretion [17–19]. Many cytokines, especially IL 1, inhibit testicular steroidogenesis [19]. In our study we demonstrated that leptin administration reduced the inhibitory role of LPS in testosterone secretions. It is known that testosterone decreases cell and humoral-mediated response [19] and the facts of higher serum testosterone and corticosterone secretion can be indicative of anti-inflammatory role of leptin.

We conclude that leptin modulates acute inflammation through intensity of LPS-induced corticosterone secretion and decreases the inhibiting role of LPS in testosterone secretion. Lack of change in serum proinflammatory and anti-inflammatory cytokine concentrations indicates that leptin modulates the endocrine system through other mechanisms at the time of acute inflammation.

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