Leptin and leptin soluble receptor changes after pulmonary endarterectomy. Relations to cortisol and cytokine network.

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Running title: Leptin and cytokines after pulmonary endarterectomy

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Summary

Objective. Leptin is a hormone that regulates food intake. During inflammatory status, leptin may contribute to the anorexia and cachexia of infection. Pulmonary endarterectomy was used as a model of non-infectious cytokine network hyperstimulation. Leptin and soluble leptin receptor (SLR) were compared with evolution of cortisol and inflammatory cytokines.

Methods. 22 patients with chronic thromboembolic pulmonary hypertension treated with pulmonary endarterectomy using cardiopulmonary bypass and deep hypothermic circulatory arrest. Leptin, SLR, cortisol, IL-1β, IL-6, IL-8, and TNFα arterial concentrations were measured before/after sternotomy, last DHCA, separation from bypass, 12, 18, 24, 36, and 48h after sternotomy.

Results. Mean duration of CPB was 338.2 min.; mean circulatory arrest time 39.9 min. The initial decline of leptin, SLR, TNFα, IL-6, and IL-8 was followed by an increase culminated 6–24 h after sternotomy. Leptin peak levels were detected +24h (28.0 ng/ml, 21.9–37.6). IL-6 culminated after separation from CPB, IL-8 was highest 12h after sternotomy. Leptin concentrations correlated with IL-6 (r=0.82), and TNFα (r=0.73).

Discussion. Large cardiovascular surgery caused a significant increase in serum leptin, which demonstrated an acute regulation by stress factors. This effect may be secondary to the inflammatory response mediated via cytokine stimulation. Correlation between leptin and IL-6 indicates the role of IL-6 in leptin induction.

Keywords: Leptin, Soluble leptin receptor, Interleukin-6, Pulmonary endarterectomy, Tumor necrosis factor-α.

Introduction

Leptin, the product of the *ob* (obese) gene, is a 16 kD protein consisting of 146 amino acid residues. Leptin was initially described as an adipocyte-derived signaling factor that, after interaction with its receptor, induced a complex endocrine and metabolic responses including control of body weight and energy expenditure (Cakir *et al.* 2007, Cirmanová *et al.* 2008, Sirotkin *et al.* 2008). More recently, it was reported that leptin seems, in addition to its role in metabolic control, to have important roles in acute phase response (Křemen *et al.* 2006, Park *et al.* 2007). During inflammatory status plasma leptin may contribute to the anorexia and cachexia of infection. Leptin may also play an important role in regulating the hypothalamo-pituitary-adrenocortical axis (Kain *et al.* 1999), angiogenesis and immune response.

Although body fat content is the major determinant of circulating leptin levels in rest period, other factors must contribute during inflammatory status (Maruna *et al.* 2006). Our previous study reported high leptin concentrations in patients with sepsis and diminished correlation of leptin and BMI in this period (Maruna *et al.* 2001). The potential role of proinflammatory cytokines in leptin regulation is supported by experimental findings. Limited studies have examined the possibility that tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6) could influence endocrine functions of adipose tissue, such as leptin production (Trujillo *et al.* 2004). Stress-induced rise in cortisol synthesis is another factor under consideration (Elimam *et al.* 1998).

In this study, endarterectomy of the pulmonary artery (PEA) was used as a model of non-infectious cytokine network hyperstimulation. PEA is a potentially curative treatment method for selected patients with chronic thromboembolic pulmonary hypertension (CTEPH), whose prognosis would be otherwise very poor. PEA provides a significant survival advantage, compared to the natural prognosis of CTEPH, medical treatment or transplant. Cardiac surgery leads to a more pronounced activation of cytokines in relation to other surgical procedures as was documented in our previous studies (Chachkhiani *et al.* 2005, Lindner *et al.* 2007). PEA is associated with hemodynamic instability in the perioperative course, suggesting the involvement of circulating mediators and cytokines as IL-1β and IL-6 (Comini *et al.* 2005, Langer *et al.* 2004).

The aims of this prospective study were, therefore, to characterize the time course of circulating leptin and soluble leptin receptor (SLR) in first 48 h after uncomplicated PEA, and to characterize the possible differences in serum leptin dynamics in relation to main inflammatory cytokines and cortisol. The plasma levels of leptin, SLR, IL-1β, IL-6, IL-8, TNFα, and cortisol were measured in patients during first 48 h after the PEA procedure.

Material and methods

The prospective study was realized on the 2nd Department of Surgery - Department of Cardiovascular Surgery of the 1st Faculty of Medicine in Prague from January 2005 to February 2008. The ethical committee of the institution approved a study protocol and informed consent was obtained from the subjects.

Patients. 22 patients - males with CTEPH (consecutive series of patients, mean age being 51.7 ± 10.8 yr., BMI 25.3 ± 3.6, mean ± SD) with New York Heart Association (NYHA) class 3.4 ± 0.4, were follow with PEA. Their mean pressure in the main pulmonary artery was 52 mm Hg. The reference group for estimation of reference laboratory values consisted of 22 healthy persons (men, the average age 48.9 ± 8.0 yr., BMI 24.3 ± 3.2). Subjects with diabetes mellitus or hypertension were excluded form the study.

Relevant gender differences in leptin concentrations with elevated leptin levels in women have been reported. To eliminate this factor, both patients and controls of one gender were included in our study.

Surgical procedures. Following median sternotomy, cardiopulmonary bypass (CPB) was established with cannulation of the ascending aorta and the inferior and superior vena cava (commonly at 7:30 a. m., according to a standard time schedule). Cooling began immediately using CPB cooling blankets, cooled to a bladder temperature of 18 - 20° C. Cardiac arrest was induced after aortic crossclamping by infusion of cardioplegic solution (mostly St. Thomas).

Approach to the pulmonary artery had to be bilateral; both pulmonary arteries had to be substantially involved. Pulmonary artery was opened; a correct dissection plane was made and pursued to the segmental branches of pulmonary artery. For precision visualization during peripheral dissection, repeated periods of deep hypothermic circulatory arrest (DHCA) with reestablishment of CPB between them were necessary. After rewarming period, patient was weaned from CPB by the stepwise reduction of pump flow. Before the end of CPB, we used an ultrafiltration of diluted blood was used for hemoconcentration. Arterial blood pressure was continuously recorded after catheterization of a femoral artery. Hemodynamic monitoring included a surgically placed left atrial catheter in all patients and flow–directed Swan-Ganz-catheter in the PEA patients.

The standard time schedule assumed the start of surgery (sternotomy) at 8:30 a. m. The procedure finished by weaning from CPB at around 4 p. m. Liquid enteral nutrition was started in all patients from 2nd postoperative day with energy intake of 1000 kcal per day (Isosource Standard, Novartis Nutrition GmbH, Osthofen, Germany). Intravenous infusions

of saline and 5% glucose were given to correct volume and ionic dysbalance during the whole tested period.

Blood Samples Collection. Arterial blood samples were drawn from femoral artery catheter before operation, after sternotomy, after the last DHCA, after separation from bypass, 12, 18, 24, 36, and 48 h after start of surgery. Blood samples of control group were drawn only at baseline. For all measurements, 5-ml of arterial blood was taken into a vacutainer tube and immediately centrifuged at 5000 rpm for 15 min. Plasma was stored at -80°C until analysis.

Leptin, Soluble Leptin Receptor, Cortisol and Cytokine Analysis. Plasma levels of both leptin and soluble leptin receptor were detected by EIA tests (BioVendor Laboratory Medicine GmbH, Heidelberg, Germany) in duplicates. All samples were within the linear detection range. Plasma concentrations of cortisol (RIA, Orion Diagnostica, Espoo, Finland), IL-1β, IL-6, IL-8, and TNFα (EIA, Immunotech, Paris, France) were measured in duplicates, too. The intra-assay coefficients of variance were below 5%.

Statistical Analysis. Data were analyzed with the statistical package for social sciences, version 12.0 (SPSS Inc., Chicago, IL, USA). Analysis of covariance (ANCOVA) was used for statistical evaluation. The normal distribution of all data was examined using the Kolmogorov-Smirnov normality test to determine subsequent use of tests for statistical comparison. As variables were not normally distributed, the data were reported as median and interquartile range. Correlation between the indicators was evaluated by the Pearson's correlation coefficient and the Spearman's rank correlation. For all the tests, $p < 0.05$ was defined as statistically significant.

Results

Mean duration of CPB was 338.2 ± 44.4 min.; mean duration of crossclamping time was 126.5 ± 20.5 min. and circulatory arrest time 39.9 ± 7.8 min. Extracorporeal circulation time was 338.2 ± 44.4 min.; duration of mechanical ventilation was 51.3 ± 36.3 h. PEA significantly decreased the mean pressure in the main pulmonary artery (mPAP) and pulmonary vascular resistance (PVR) and increased cardiac index (CI) within first 24 h after surgery (**Table 1**).

Postoperative course of all tested patients was uncomplicated within 48 h after surgery. One patient died 9th day after PEA with the diagnosis of bronchopneumonia. The first clinical signs of inflammation were found 8th day after PEA. Following section proved this diagnosis. The only patient with poor prognosis didn't differ from uncomplicated course in both leptin and cytokine dynamics in evaluated 48-h period after sternotomy.

The mean preoperative leptin plasma levels were 4.12 ng/ml (interquartile range, 3.19 – 5.22), and leptin of all tested patients was below 7.5 ng/ml 24 h before surgery. Preoperative SLR levels were 36.8 ng/ml (32.1 – 41.8). The mean preoperative IL-6 plasma concentrations were 21.4 ng/ml (14.1 – 31.5). There was no correlation between preoperative plasma levels and hemodynamic parameters as well as between IL-6 and hemodynamic status. All tested inflammatory parameters including IL-1β (4.8 ng/l, 1.2 – 7.7), TNFα (31.0 ng/l, 18.6 – 44.9), and IL-8 (85.0 ng/l, 38.2 – 128.9) didn't differ from reference group 24 h before start of surgery.

Arterial blood samples analysis documented a transient initial decline of leptin (minimum 3 h after sternotomy, $p < 0.01$ to preoperative levels) with subsequent elevation (**Fig. 1**). Transient initial decline of leptin correlates significantly with decrease of hematocrit during hemodilution ($r = 0.76$, $p < 0.01$). Serum leptin levels increase postoperatively from +3 h reaching a peak level 24 h after sternotomy (28.0 ng/ml, 21.9–37.6). Leptin levels were elevated in all tested patients 24 h after sternotomy in comparison to preoperative levels. Peak levels were statistically significant higher in relation to both preoperative levels and control group ($p < 0.001$).

SLR dynamics is shown on **Fig. 2**. After start of surgery, there was a transient decline of SLR levels. Minimal SLR levels attained 3 h after sternotomy (13.7 ng/ml, 11.9 – 15.0) differed significantly from preoperative concentrations on $p < 0.01$. Similarly as leptin decline, SLR decrease correlated well with a course of hematocrit during hemodilution ($r = 0.79$, $p < 0.01$). SLR concentrations reverted to initial levels 12 h after start of surgery.

The same initial decrease was revealed for IL-6 (**Fig. 3**), IL-8, and TNFα, too. Peak levels of IL-6 were reached 6 h after CPB (524.2 ng/l, 418.0 – 668.2) as well as TNFα culmination (154.2 ng/l, 97.2 – 228.3). IL-8 culminated later, 12 h after sternotomy (438.0 ng/l, 264.2 – 644.9). IL-1β elevation with maximum 6 h after start of surgery wasn't statistically significant in relation to initial levels.

Preoperative plasma cortisol levels measured by RIA were 411.6 ng/l (318.1-485.2). Without initial decrease shown in other parameters, cortisol concentrations culminated 6 h after sternotomy (1462.7 ng/l, 1102.6 – 1879, $p < 0.001$ in relation to both reference group and preoperative status). Despite following decrease, cortisol remained elevated 48 h after start of surgery (**Fig. 4**).

Postoperative peak values of leptin and IL-6 correlated closely ($r = 0.82$, $p < 0.01$). Significant correlation was revealed for peak values of leptin and TNFα ($r = 0.73$, $p < 0.01$), too. Correlation between leptin and other cytokines wasn't significant on $p < 0.05$. No correlation was found between plasma cortisol and leptin levels. There was diminished

correlation of plasma leptin and BMI during first 24 h after surgery: $r = 0.775$; $p < 0.01$ before operation, $r = 0.48$; $p < 0.05$ in samples +24 h after start of surgery. No correlation was found between plasma leptin levels and mean arterial blood pressure or between plasma leptin and creatinine levels in postoperative period.

No significant correlation was revealed between SLR concentrations and tested inflammatory parameters on $p < 0.05$.

Discussion

Presented results demonstrate the evolution of leptin and SLR in relation to a large cardiovascular surgery with deep hypothermia and circulatory arrest. It has been previously shown that leptin change postoperatively (Křemen et al. 2006, Maruna *et al.* 2005) and leptin evolution is related to inflammatory status. Cardiac surgery leads to a more pronounced activation of cytokines than that some other surgical procedure (Dörge *et al.* 2003). This cytokine 'burst' mediates a systemic response by the body's inflammatory system, well known as the systemic inflammatory response syndrome (SIRS) (Giamarellos-Bourboulis *et al.* 2004). Therefore PEA represents a suitable clinical model of cytokine network hyperstimulation without contribution of infectious factor.

Several factors may influence the evolution of serum leptin levels after cardiac surgery in the absence of postoperative complications. The combination of CPB, local trauma, deep hypothermia, and pulmonary and myocardial reperfusion leads to substantial changes in the immune system. Our findings are consistent with the hypothesis that cytokine network influences the leptin secretion. Positive correlation between leptin and IL-6, as well as between leptin and TNFα indicates the role of both cytokines in leptin induction in perioperative phase.

Trujillo *et al.* (2006) date suggest that high systemic TNF may contribute to increased leptin production during stress. TNFα and glucocorticoids synergistically increase leptin production in human adipocytes. Synergistic effects of local or systemic TNF or IL-6 in combination with glucocorticoids may contribute to increased leptin expression in response to surgical stress as was seen in our study. Presented study did not address the mechanism of the interaction of TNFα, IL-6 on leptin production. However, it appears that both cytokines increase leptin most markedly in the presence of corticoids. From a physiologic point of view, synergistic effects of glucocorticoids and IL-6 on leptin suggest a role in stress-induced increases in leptin production (Trujillo *et al.* 2004).

Plasma levels of cortisol increased in tested group, however without significant correlation to leptin levels. Glucocorticoids are known to up-regulate serum leptin, and are reported to

amplify the *in vitro* up-regulation of leptin by other factors (Elimam *et al.* 1998). This is hypothesized that the rise in serum cortisol observed after surgery enhanced the effect of inflammatory factors. The effects of cytokines on leptin in man are still controversial. Unchanged serum leptin levels after high doses of prednisolone given to healthy volunteers, have also been reported (Tataranni *et al.* 1997), and it has been questioned whether the effects of glucocorticoids on leptin in humans are restricted to acute pharmacological dosing.

Even high cortisol values observed in our patients did not prevent the activation of TNFα and IL-6. Cortisol is the major regulator of the expression and action of pro-inflammatory cytokines limiting the amplitude and duration of SIRS. Via the release of the anti-inflammatory cytokines as IL-10, cortisol prevents indirectly the further synthesis of pro-inflammatory mediators. The time course of pro-inflammatory cytokines after surgery is thus determined by the interaction of stimulatory factors (tissue trauma, CPB, hypothermia) and endogenous inhibitors on both local and systemic levels.

Significant correlation between leptin and IL-6 was found in our study. Park *et al.* (2007) recently demonstrated the same relation between leptin and IL-6 in other model of non-infectious inflammatory reaction - ankylosing spondylitis. Serum leptin levels were increased and significantly associated with IL-6 levels. Both indicate that IL-6 can be a regulator of leptin generation in early postoperative period. As was described by Trujillo *et al.* (2004), IL-6 had no significant effects *in vitro* under basal conditions, the combination of IL-6 and dexamethasone, compared with dexamethasone alone, increased leptin production two-fold. It is possible that pro-inflammatory cytokines induce *ob* gene transcription *in vivo* via secondary mediators such as transforming growth factor β (Granowitz 1997).

Results document that leptin culmination is delayed in alignment to pro-inflammatory mediators as IL-6 and TNFα. Both mentioned cytokines are assumed to activate leptin induction *in vivo*. The following decline of leptin levels 48 h after surgery corresponds to the absence of a further insult that may induce more leptin production. Lacking correlation between leptin and IL-1β plasma levels in our patients may be explained by prevailing local activities of this cytokine. Plasma 'overflow' of IL-1β and other cytokines during SIRS doesn’t reflect their tissue expression directly. Especially IL-1β showed only mild elevation bellow statistical significance in postoperative period.

The main limitation of our study is a potential role of nutritional factors in leptin dynamics. The changes observed in leptin concentrations could be affected by the reduced food intake in our patients. To eliminate this factor may be difficult in clinical conditions. Both experimental and clinical studies showed that fasting inhibits leptin production. We suppose that short periods of fasting and surgical stress can sensitize the leptin response to

stimulatory factors. In summary, both short-term fasting and stress-related cortisol elevation might constitute a background for leptin reactivity to inflammatory mediators. Trujillo *et al.* (2004) experimental results supported this conception.

Leptin receptor was found to be a member of the class I cytokine receptor family. Alternate splicing from a single gene derives the six isoforms of both membrane bound and soluble receptors. SLR levels are indirectly proportional to adiposity and are increased in females versus males. Many different physiological and pathophysiological conditions, e g. adiposity, sex steroids, and leptin independently regulate plasma SLR. Soluble receptors control the amount of free leptin and the rate of leptin clearance (Zastrow *et al.* 2003). Therefore changed SLR levels may modulate the actions of leptin in tissues (Cohen *et al.* 2007).

Our date showed that SLR is not an acute phase reactant in postoperative period. Voegeling *et* Fantuzzi (2001) reported increased levels of SLR in the state of inflammation in mice. Other studies did not support their findings, and this question was not clarified, yet. Schoff *et al.* (2003) demonstrated no influence of surgical stress on postoperative soluble leptin receptor plasma levels and their finding are in conformity with our results.

Transient initial decline of both leptin and SLR after surgery - statistically significant on $p < 0.01$ - is explained mostly by hemodilution. Significant correlation between leptin and hematocrit demonstrated this phenomena. We suspect the role of hemofiltration as another factor affecting biphasic leptin course in perioperative phase, too. Hemofiltration is used for hemoconcentration at the end of the operation. This biphasic postoperative course was not limited to leptin and SLR. The tendency to transitory decrease after start of surgery was revealed in all tested parameters, but without statistical significance on $p < 0,05$ excepting IL-6.

Leptin is structurally similar to granulocyte colony-stimulating factor, member of IL-6 cytokine family. It has been reported that leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells and can enhance the proliferation and phagocytic activity of macrophages (Loffreda *et al.* 1998, Santos-Alvarez *et al.* 1999). These results identify an important and novel function for leptin: up-regulation of inflammatory immune responses. However its physiological importance in acute phase reaction remains unclear. Induction of leptin during the surgical stress response may contribute to the wound healing, anorexia, and activation of hematopoiesis. These and other effects were reported in experimental studies.

In summary, large cardiovascular surgery caused a more than sixfold increase in serum leptin. Postoperative leptin culmination was delayed in alignment to pro-inflammatory

mediators. The positive correlation between leptin and IL-6 indicate that mentioned cytokine plays a role in leptin induction, and this mechanism is similar to other models of SIRS-induced cytokine network.

Acknowledgement

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Abbreviations

BMI	body mass index
CI	cardiac index
CPB	cardiopulmonary bypass
CRP	C reactive protein
CTEPH	chronic thromboembolic pulmonary hypertension
DHCA	deep hypothermic circulatory arrest
IL	interleukin
mPAP	main pulmonary artery pressure
PEA	pulmonary endarterectomy
PVR	pulmonary vascular resistance
SIRS	systemic inflammatory response syndrome
SLR	soluble leptin receptor
TNFα	tumor necrosis factor-α

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Table 1. Hemodynamic status preoperatively and early after operation

	Preoperative	Postoperative (24 h)	*p* value
mPAP (mm Hg)	58.0 ± 11.7	25.7 ± 7.27	< 0.001
CI ($l.min^{-1}m^{-2}$)	1.8 ± 0.25	2.99 ± 0.43	< 0.001
PVR ($dynes.s.cm^{-5}$)	1161.5 ± 306.5	201.9 ± 99.8	< 0.001

Abbreviations: mPAP - mean pressure in the main pulmonary artery; CI - cardiac index; PVR - pulmonary vascular resistance.

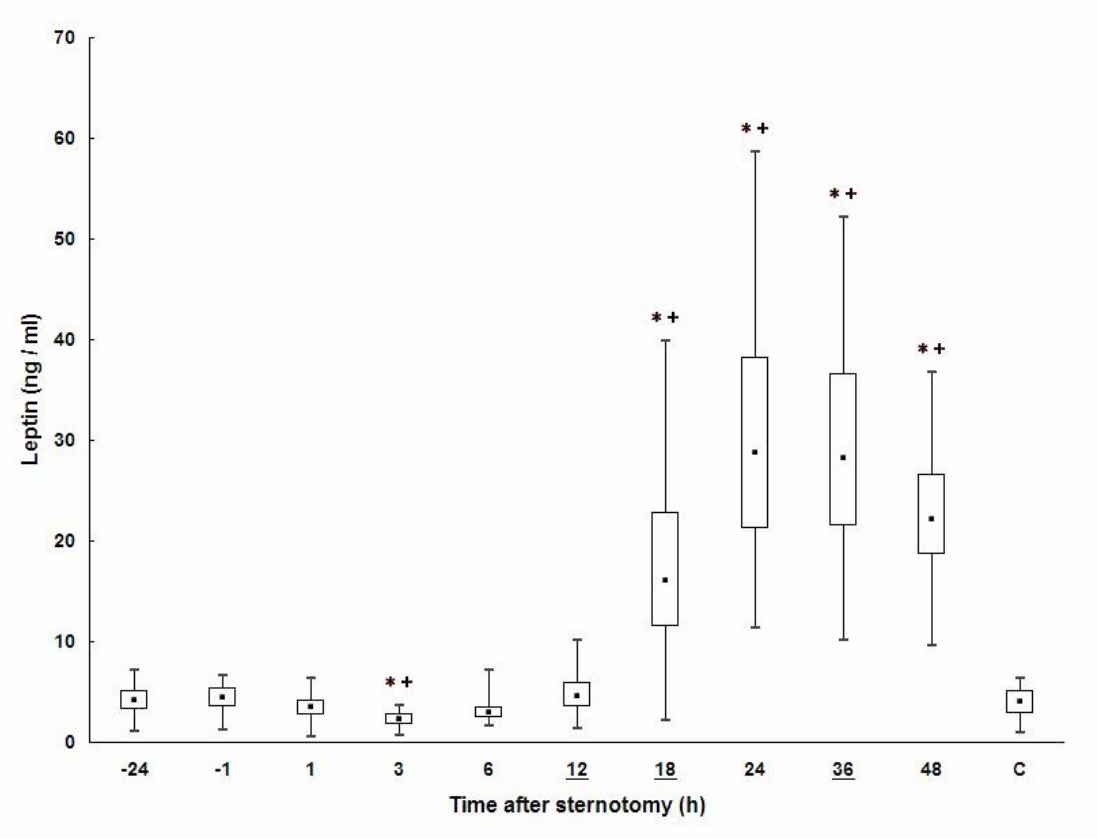

Fig. 1. Leptin dynamics in perioperative period

The same setting used for Fig. 1 – 4:

Box and whisker plot depicting the median values, interquartile range and full range.

C = Control group

Underlined times correspond to samples taking in a dark period.

∗ … Statistically significant differences to control group on p ‹ 0,05.

+ … Statistically significant differences to preoperative values on p ‹ 0,05.

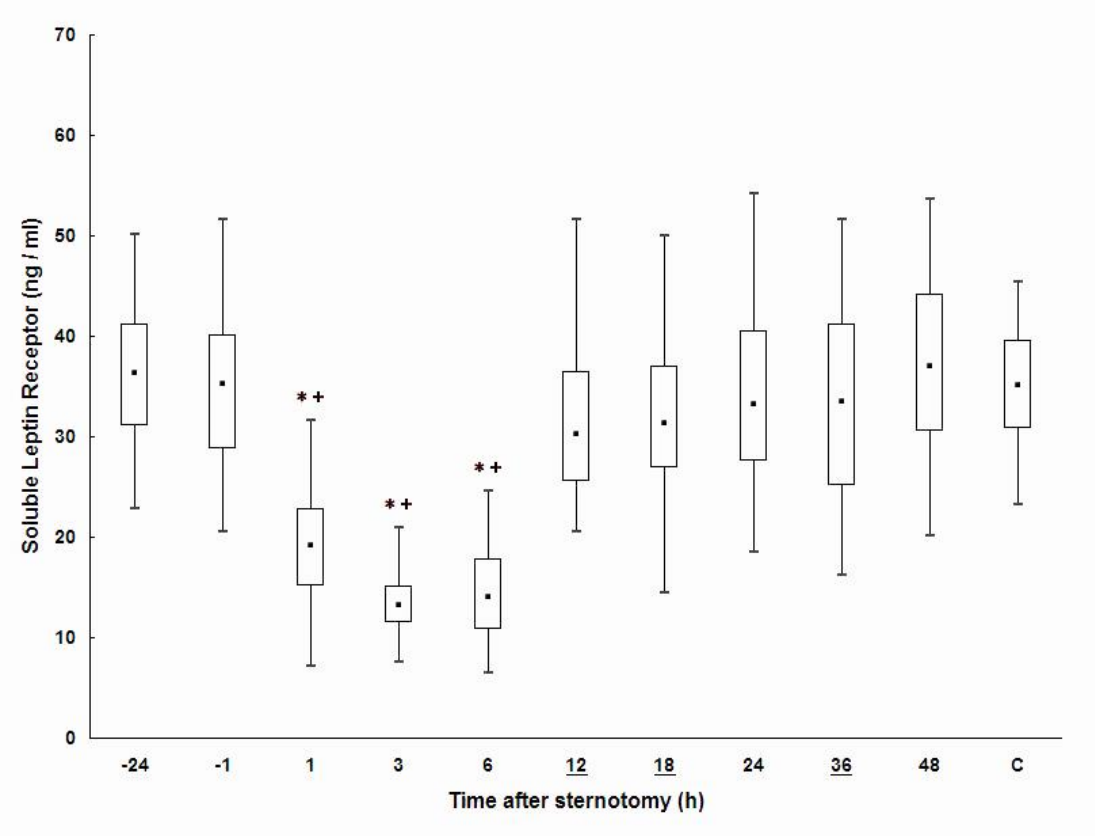

Fig. 2. Soluble leptin receptor dynamics in perioperative period

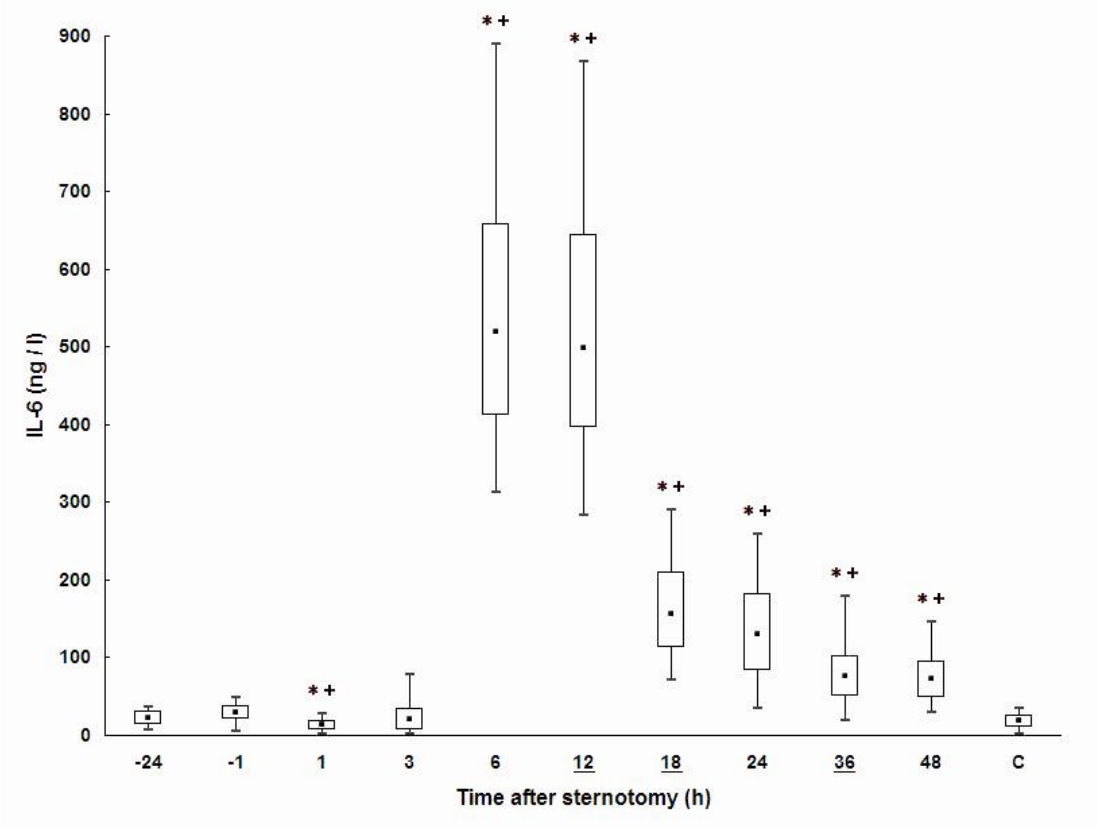

Fig. 3. IL-6 dynamics in perioperative period

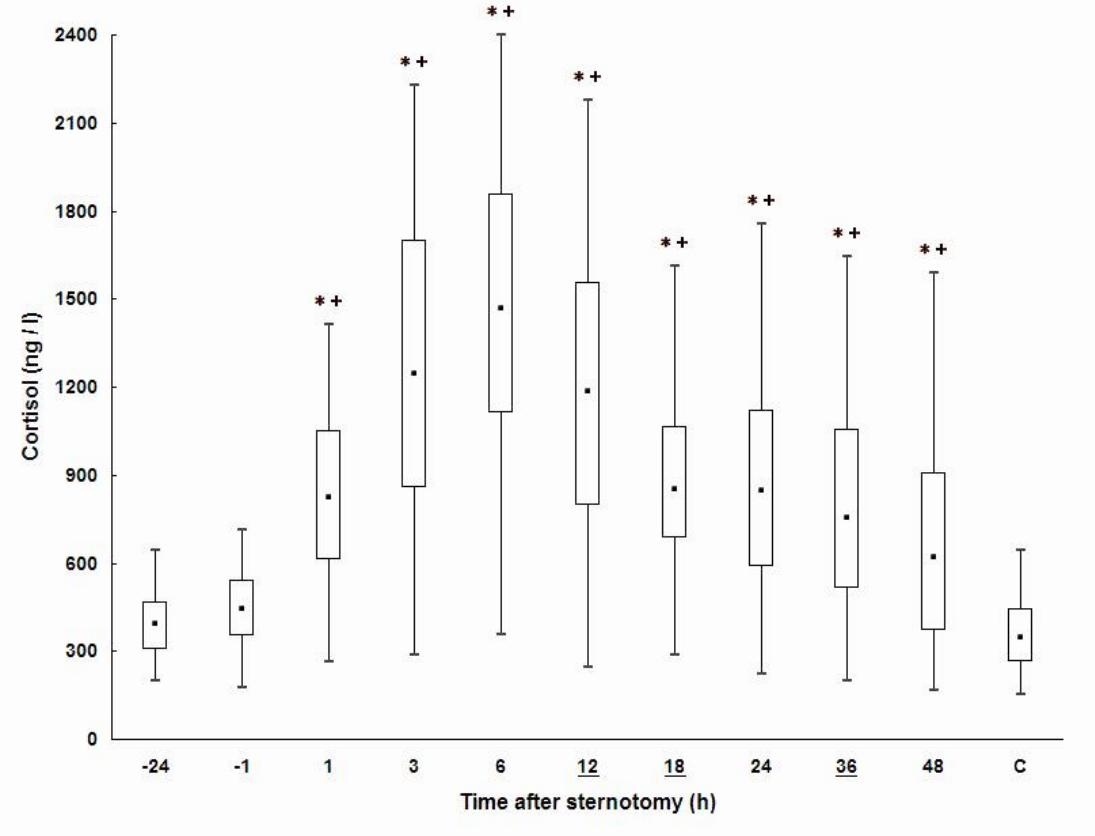

Fig. 4. Plasma cortisol dynamics in perioperative period