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Adipocytokine: A New Family of Inflammatory and Immunologic Markers of Invasiveness in Major Urologic Surgery

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Abstract

Background: Laparoscopic surgery has been proposed to reduce surgical trauma and diminish patients' stress response.
Objective: To investigate the role of the adipocytokine, in combination with changes in other known inflammatory markers, in patients undergoing radical prostatectomy.
Design, setting, and participants: A total of 580 patients were enrolled in this prospective study. Laparoscopic extraperitoneal radical prostatectomy (LRP) was performed in 286 patients, and open retropubic radical prostatectomy (RRP) in 294 patients.
Intervention: Blood samples were collected preoperatively and up to 5 d postoperatively.
Measurements: Serum concentrations of acute phase markers, interleukins (IL), and the adipocytokine leptin were measured at each time point by means of enzyme-linked immunosorbent assay. Clinical data were collected and analysed.
Results and limitations: Patients undergoing LRP had significantly lower IL-6 and adipocytokine levels at all measurement time points. However, biphasic kinetics of adipocytokine serum levels were observed during the postoperative course in all patients. LRP was associated with less adipocytokine and IL-6 release, indicating a smaller degree of surgical insult and the minimal invasive nature of this procedure. The limitation of this study was its nonrandomised design.
Conclusions: Adipocytokines might serve as additional immunologic markers of invasiveness in major urologic surgery.

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1. Introduction

Major urologic surgery procedures cause a systemic inflammatory reaction with tissue damage due to surgical trauma and anaesthesia-related interventions. This leads to local activation of various cells (eg, monocytes and macrophages), which release cytokines and other mediators [1]. This activation may be followed by a systemic acute-phase reaction, which is proportional to the extent of tissue damage [2]; however, tissue damage from abdominal

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surgery may also result in postoperative fatigue and affect various organ systems with possible organ failure [1,2].

The advent of laparoscopic surgery has greatly influenced urologic surgery. It is thought to preserve immune function after surgery due to smaller incisions, reduction in tissue injury, and less blood loss [2]. However, decreased perioperative stress is particularly important when performing oncologic surgery, as exaggerated activation or reactive suppression of the immune system might affect tumour growth and dissemination.

Physiologic response to acute stress, as induced by major surgical interventions, involves finely integrated interactions between the autonomic nervous system, the endocrine system, the immune system, and metabolism. In particular, recent research has focused on the activation of endocrine stress response, which includes the activation of the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic medullary system, and their modulators [3]. Moreover, recent investigations have also implicated the circulating levels of adipocyte-derived hormones—the so-called adipocytokines—such as leptin, which change acutely under some stressful conditions in rodents and humans [4,5]. In particular, leptin appears to have a role in the cross-talk between adipose tissue and the immune system, as well as the HPA axis [6].

During the last few years, our group has been intensively engaged in evaluation of invasiveness of open and laparoscopic urologic procedures in terms of activation of immunologic and stress-hormonal responses [7,8]. In the present study we sought to investigate the role of a new family of inflammatory markers, the adipocytokines—specifically, leptin—in combination with changes in perioperative release of other known inflammatory markers and cytokines in patients undergoing laparoscopic radical prostatectomy (LRP) and open radical retropubic prostatectomy (RRP). The null hypothesis of this study was that, due to reduced surgical trauma and the minimally invasive nature of the laparoscopic procedure, less activation of adipocytokine system and inflammatory markers might be expected. The secondary aim was to explore the association between leptin, other known mediators of systemic inflammation, and the clinical course in order to determine whether this adipocytokine could serve as an additional marker in assessing the invasiveness of major urologic surgeries.

2. Patients and methods

This was a prospective, nonrandomised, controlled, single-centre study including 580 patients recruited between January 2004 and December 2008. The study was approved by the institutional review board. Written informed consent was obtained from all patients. LRP (group 1) was performed in 286 patients and 294 patients underwent RRP (group 2). The choice between LRP and RRP was based on a joint decision by the patients and physicians, and all the patients were theoretically suitable for the other surgical approach.

2.1. Study protocol

Patients were recruited on the day before surgery and venous blood was drawn for baseline measurements of biochemical serum parameters as well as plasma leptin and cytokine levels. All baseline blood samples were collected in ethylenediaminetetraacetic acid-containing tubes in the morning between 0900 and 1200 (time point: T_{BL}). After the operation, all patients were transferred to the ward and serial blood samples were collected from the venous line at 5 h postoperatively (time point: T_1), as well as 24 h, 48 h, and 5 d after surgery (time points: T_2–T_4, respectively). Plasma was recovered immediately from all samples, aliquoted, and frozen at −80 °C until final use. Patients with major postoperative complications (eg, revision or perioperative surgically treated lymphocele) were excluded from the serum inflammatory hormone analysis.

2.2. Anaesthesia and surgical procedures

Total intravenous general anaesthesia was induced and maintained with sufentanil and propofol in all groups. No anaesthetic gases were used. After relaxation with pancuronium bromide, the trachea was intubated, and controlled normocapnic ventilation with an air-oxygen mixture was started. No patients received corticosteroids before, during, or after the operation. The techniques of LRP and RRP were described in detail elsewhere [8,9].

2.3. Measurements

Clinical variables included the duration of surgery, intraoperative blood loss, blood transfusion rate, complication rate, duration of hospitalisation, and duration of urinary catheterisation. Oncologic variables evaluated were Gleason score, pathologic stage, and surgical margin status. As part of the routine clinical observance, selected clinical and biochemical data were recorded at each time point. Two acute-phase proteins, C-reactive protein (CRP) and serum amyloidase antibody (SAA), were determined using highly sensitive immunonephelometric assays (Dade Behring Co, Marburg, Germany). Leptin and inflammatory cytokine analyses were performed as follows: Samples were assayed in a single large batch, duplicates agreed within 15%, and quality assessment samples were within the manufacturer's defined range. Enzyme-linked immunosorbent assay techniques were applied to determine leptin levels and levels of interleukin (IL)-6 (a proinflammatory cytokine), and an anti-inflammatory cytokine, IL-10 (IBL, Hamburg, Germany). The intra- and interassay coefficients of variation were 4.1–5.4% and 3.6–7.8% for leptin, <10% and 7.4% for IL-10, and 5.2% and 3.4% for IL-6, respectively. The plasma hormone and cytokine concentrations at each time point (T) were corrected for haemodilution according to the formula:

The mean correction factors plus or minus SD were 1.15 ± 0.21 for group 1 and 1.28 ± 0.42 for group 2, respectively.

$$Marker\ level\,(corrected) = \frac{Marker\ level\,(measured) \times haematocrit\,(baseline)}{heamatocrit\,(T)}$$

2.4. Statistical analysis

Statistical analysis was performed using SigmaPlot software v. 11.0 (SPSS Inc, Chicago, IL, USA). Data are expressed as mean plus or minus standard error of mean (SEM) or percent of baseline, and statistical significance was accepted at $p < 0.05$. Inflammatory marker levels were analysed by the repeated measures two-way analysis of variance (ANOVA). Repeated measurement post hoc between-groups effects were tested by Tukey test. Within-group effects for time were tested by post hoc Dunnett contrasts of baseline values versus subsequent measurements. Pearson correlation analysis was used to determine relationships between leptin, other members of the inflammatory cascade, and acute phase proteins.

3. Results

Preoperative and intraoperative patient characteristics, as well as clinical and histologic results are shown in Table 1.

Table 1 – Characteristics of study population and perioperative results*

Variable	LRP	RRP	*p*
Patients, No.	286	294	–
Age, yr			
Median	61.8	64.4	NS
Range	42–73	49–77	NS
Body mass index kg/m^2, mean ± SD	26.8 ± 2.1	27.6 ± 1.4	0.11
Preoperative PSA level, ng/ml (range)	6.19 (2.3–11.1)	6.32 (4.4–12.8)	0.23
Preoperative clinical stage, %			
T1a	2	5	0.054
T1c	75	73	0.09
T2a	19	16	0.06
T2b	4	6	0.08
Biopsy Gleason score	5.8	6.4	0.09
Operative time, min (range)	146 (115–228)	116 (81–182)	<0.05
Blood loss, ml (range)	152 (85–710)	489 (200–1980)	<0.05
Transfusion, %	3.7	8.8	<0.05
Complications, %			
Rectal lesion	1.9	1.4	0.24
Lymphocele	4.1	3.7	0.09
Wound infection	2.9	3.6	0.06
Revision	1.7	2.8	0.06
Conversion	1.1	–	0.057
Duration of catheterization, d	8.1	10.3	<0.05
Hospitalization, d	8.2	10.4	<0.05
Histopathology			
Mean prostate weight, g (range)	41.6 (20–88)	44.1 (22–120)	<0.05
Median Gleason score (specimen)	6.1	6.9	0.25
Pathologic stage, %			
pT2a	16	19	0.056
pT2b	27	22	0.053
pT2c	23	25	0.07
pT3a/b	33	34	0.13
Positive lymph nodes, %	0	2.1	0.052
Positive surgical margins, %			
pT2a/b/c	11.5	13.6	0.06
pT3a/b	31	33	0.08

* Data are presented as median.
NS = not significant; LRP = laparoscopic extraperitoneal radical prostatectomy; RRP = retropubic radical prostatectomy; PSA = prostate-specific antigen.

During the course of the study no case of perioperative mortality was observed. LRP resulted in less bleeding ($p < 0.05$), less need for blood transfusion ($p < 0.05$), faster recovery ($p < 0.05$), and less duration of catheterisation ($p < 0.05$). There were no significant differences in the oncologic outcome of patients treated by RRP or LRP in our series (Table 1). The acute-phase proteins CRP and SAA increased early after both procedures, reaching peak levels at 48 h postoperatively. There was a statistically significant difference in CRP levels at 24 h ($p < 0.05$) and 48 h ($p < 0.01$) postoperatively between LRP and RRP (Fig. 1).

3.1. *Plasma inflammatory cytokine levels*

Serum levels of the proinflammatory cytokine IL-6 increased during the operation and in the early hours after the operation, reaching peak values at 5 h postoperatively (Fig. 2a). Thereafter they declined gradually, but remained above the baseline values ≤5 d after the operation. The changes in cytokines levels over time in each group were statistically significant as evaluated by repeated-measures ANOVA ($p < 0.001$). There was also significant difference between the two groups at some time points with serum IL-6 levels being significantly lower after LRP than after RRP (Fig. 2a). Serum levels of IL-10 showed similar kinetics in both groups, with these being consistently higher in the laparoscopic group during the early postoperative phase (Fig. 2c).

3.2. *Circulating adipocytokine*

Immediately after the operation, a marked decrease in leptin serum levels occurred in all patients, reaching minimum levels of 67.03% of baseline in group 1 and 93.1% in group 2 at 5 h postoperatively ($p < 0.001$). Fig. 2b depicts the kinetics in plasma levels of leptin over time in both groups. Two-way ANOVA showed that the changes in hormone levels were quite significant within the groups over the time (overall: $p < 0.001$). However, patients undergoing RRP (group 2) had higher leptin levels at all

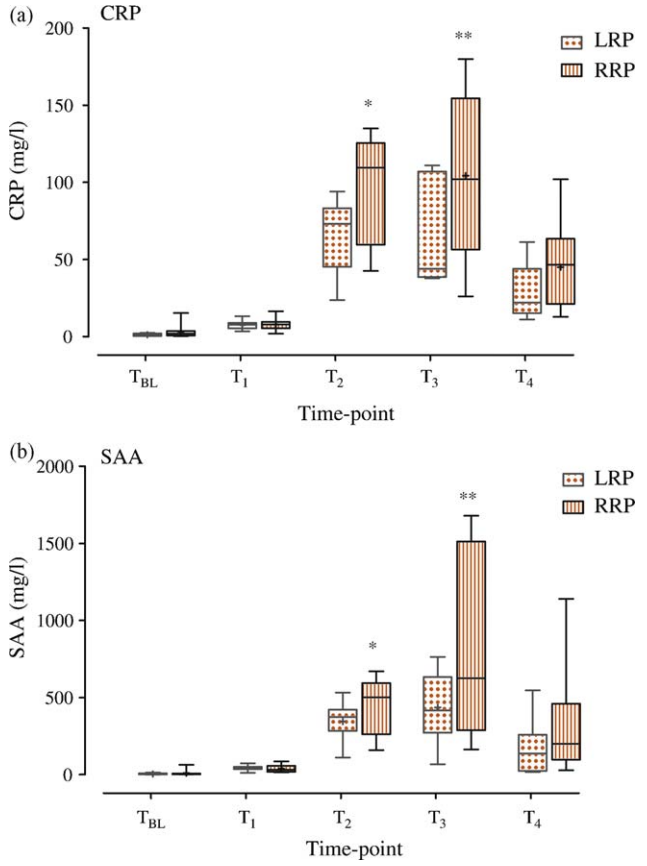

Fig. 1 – Changes in serum levels of acute phase proteins (a) C-reactive protein (CRP) and (b) serum amyloidase antibody (SAA) during the perioperative time course of 4 d after extraperitoneal laparoscopic radical prostatectomy (LRP, filled bars) and retropubic radical prostatectomy (RRP; white bars). Data are reported as median (percentiles: 5, 25, 75, and 95) and mean (shown as +). Asterisks denote significantly different values among the treatment groups.
*** $p < 0.05$.**
**** $p < 0.01$.**
***** $p < 0.001$.**

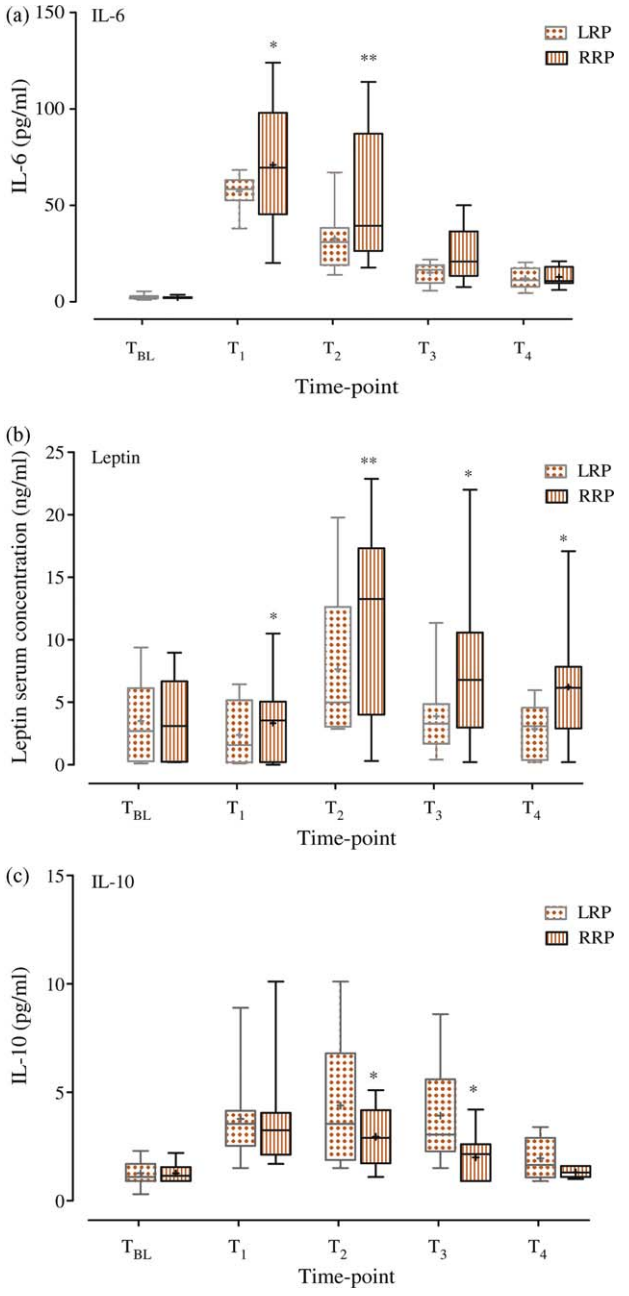

Fig. 2 – Kinetics of serum levels of (a) proinflammatory cytokine interleukin (IL)-6, (b) adipocytokine leptin, and (c) anti-inflammatory cytokine IL-10 during the perioperative time course of 4 d after extraperitoneal laparoscopic radical prostatectomy (LRP, filled bars) and retropubic radical prostatectomy (RRP; white bars). Data are reported as median (percentiles: 5, 25, 75, and 95) and mean (shown as +). Asterisks denote significantly different values among the treatment groups.
*** $p < 0.05$.**
**** $p < 0.01$.**
***** $p < 0.001$.**

measurement times compared to group 1, with this difference reaching statistical significance at all postoperative sampling times (overall: $p < 0.001$). Further, there was a significant inverse relationship between leptin and cytokines levels (Fig. 3). During the early hours of the surgery, the decrease in leptin levels paralleled the increase in IL-6 and IL-10 levels. Later, as leptin started to increase again and reached its maximum 24 h postoperatively, both cytokine levels declined gradually to preoperative levels. Furthermore, Pearson correlation analysis revealed a strong correlation between leptin, CRP, and SAA (Fig. 3). These associations were evident when correlation analysis was applied to the entire study population, and remained valid in both treatment groups.

4. Discussion

Several studies have shown diminished stress responses following laparoscopic surgery [10,11]. A relatively new player in the cascade of the human stress response is the adipocyte-derived adipocytokine leptin. Leptin is the hormone produced by transcription of the *ob* (obese) gene, primarily of the human adipocytes [12]. Recent evidence supports leptin's role in more complex physiologic systems such as angiogenesis, haematopoiesis, immunity, and

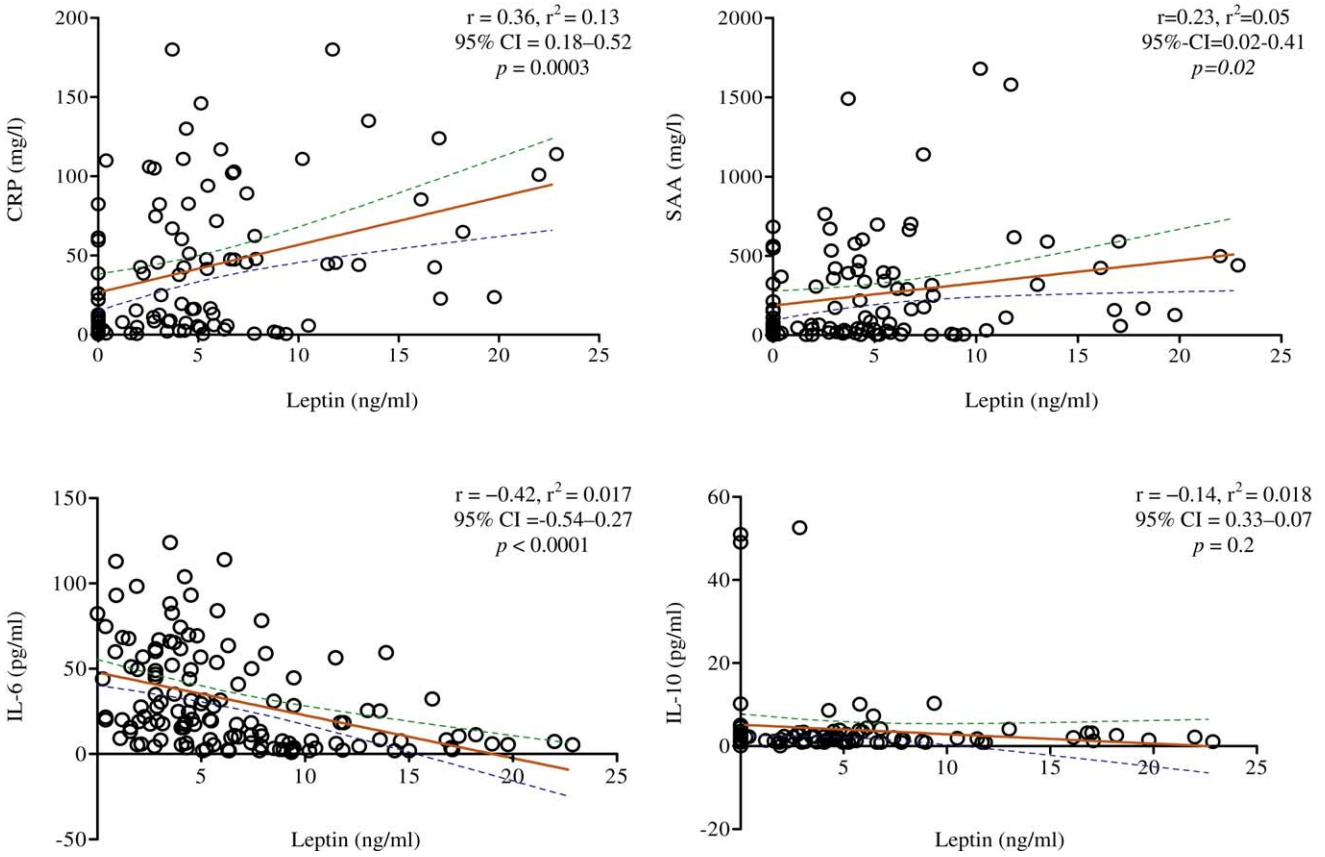

Fig. 3 – Pearson correlation analysis of serum levels of the adipocytokine leptin and other markers of systemic inflammation in patients undergoing radical prostatectomy. These associations were evident when correlation analysis was applied to the entire study population and remained valid in both treatment groups.
CRP = C-reactive protein; SAA = serum amyloidase antibody; IL-6 = interleukin-6; IL-10 = interleukin-10.

inflammation [13]. Leptin has a structure similar to that of the IL-6 family of cytokines. Both Janus kinases (JAK) and signal transducers and activators of transcription (STAT) are involved as downstream components of leptin and IL-6 signalling, which demonstrates several similarities between leptin and the IL-6 family [14].

In the present study, we evaluated the kinetics of plasma levels of leptin and other inflammatory cytokines in patients undergoing RRP or LRP. During the postoperative course of up to 5 d, a biphasic kinetic of serum leptin levels could be observed in all patients. During the operation and in the hours thereafter, a marked decrease in leptin levels occurred, reaching minimum levels 5 h postoperatively in all study patients regardless of type of operation (Fig. 2b). We hypothesise that this could be due to the effects of anaesthesia and related medications. Additionally, an effect of pre- and perioperative starvation could contribute to this acute fall in leptin levels. In clinical studies, it has been shown that during starvation, serum leptin level decreases, corresponding to the tendency of organism to conserve energy and minimise metabolism in order to survive [15].

This fall in leptin expression in response to stress may also serve other physiologic roles. First, because leptin has an inhibitory effect on cortisol secretion, it had been hypothesised that the initial stress-induced decrease in leptin levels may permit the generation of higher cortisol levels [16]. This hypothesis is supported by reports indicating that high levels of leptin inhibit both the response of the HPA axis to acute stress and the adrenal cell response to adrenocorticotropic hormone [17]. Second, leptin may act in support of the innate inflammatory response and in opposition to the adaptive immune response. Indeed, leptin has been shown to induce the production of interferon-gamma and suppress the production of antibody-inducing IL-4 in a dose-dependent manner [18].

The perioperative secretion of cytokines is the result of a number of processes that occur during surgery: immunomodulatory effects of anaesthesia and drugs administered perioperatively, tissue damage, and the likelihood of endotoxaemia. Several studies describe an increase in blood levels of IL-6 in patients undergoing major surgery [19]. IL-6 is recognised as an early and robust marker of the systemic inflammatory response following surgery. Cruickshank et al reported that the amount of IL-6 secretion in surgical patients has a positive correlation with the extent of surgical injury [20]. In accordance with our results in the urologic surgery population, they describe increased IL-6 blood levels with peak levels 6–12 h after incision in patients undergoing abdominal surgical procedures. However, peak levels of IL-6 were found in our study participants

also in the postoperative period. Postoperatively, on the day of surgery, IL-6 was maximally elevated compared with the preoperative values. Experimental animal and clinical human studies have provided further evidence of a link between proinflammatory cytokines and leptin [13,21]. However, in the present study we observed an inverse relationship between plasma levels of leptin and IL-6 (Fig. 3). The plasma levels of this cytokine rose early postoperatively, reached their maximum levels after 24 h, and declined gradually thereafter, as leptin levels were about to increase. However, the nature of the interaction between leptin and the cytokine system is currently not fully understood and requires further investigation [22–25].

Analysis of our data has further revealed that differences in the methods used for surgery (RRP vs LLP) are reflected in the leptin and cytokine time course. We observed that RRP patients had higher leptin levels at all measurement times than LRP patients, with the differences reaching statistical significance at some points. Considering the interactive relationship between leptin and several members of cytokine family, these findings provide further evidence that laparoscopy has a reduced impact on human physiology compared with open surgery. Previous studies have shown similar changes in circulating leptin levels after different types of surgery [26]. Karayiannakis et al showed that the cumulative response of leptin to surgery correlated significantly with both IL-1a and IL-6 responses, and was significantly lower after laparoscopic cholecystectomy than after open cholecystectomy [27]. Kain et al measured plasma leptin levels in 21 women before, during, and after total abdominal hysterectomy [28]. They reported that leptin levels were maximally decreased at 2 h after surgery, which was followed by an increase to just above preoperative values at 24 h postoperatively. In our study, similar kinetics in serum IL-6, IL-10, and adipocytokine concentrations could be observed in both groups, with postoperative levels being consistently lower after LRP than after RRP.

5. Conclusions

Radical prostatectomy is associated with acute perioperative changes in plasma levels of the neurohormonal stress adipocytokine leptin, as well as inflammatory cytokines, thereby affecting the activation of the immune system and the clinical course. Adipocytokine could serve as new marker of invasiveness in major urologic surgery.

Author contributions: Francesco Greco had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Greco, Hoda.
Acquisition of data: Greco, Wagner, Hoda, Inferrera, Fischer.
Analysis and interpretation of data: Greco, Wagner, Hoda, Fischer, Reichelt.
Drafting of the manuscript: Greco, Hoda.
Critical revision of the manuscript for important intellectual content: Fornara, Greco, Hoda.
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