

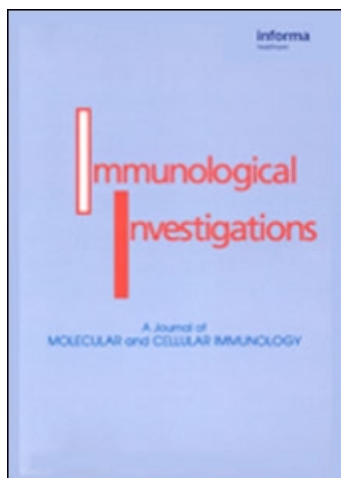
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Serum Leptin Levels Depend on Allergen Exposure in Patients with Seasonal Allergic Rhinitis

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Several studies have outlined a possible relationship between an increased body mass index (BMI) and respiratory allergic diseases, such as asthma and rhinitis. There are conflicting data about the role for leptin in allergic rhinitis. Therefore, the aim of the study was to evaluate the serum leptin levels in patients with seasonal allergic rhinitis (SAR), enrolled during and outside the pollen season, and in a group of healthy controls. The study included 137 subjects; 62 symptomatic SAR patients evaluated in season, 41 symptomless SAR patients out season, and 34 normal subjects. All subjects were consecutively evaluated. A skin prick test and blood sampling for assessing serum leptin levels were performed in all subjects. After analysing genders separately, symptomatic male patients had significantly higher levels than symptomless and normal subjects ($p = 0.0004$ and 0.0031 respectively), symptomatic female patients showed significantly higher levels than normal females ($p = 0.0002$). This study provides the evidence that leptin serum levels depend on allergen exposure in SAR patients.

Keywords Adipose tissue, Serum leptin, Allergic rhinitis, Pollens.

INTRODUCTION

Obesity and weight gain have been associated with an increased risk for respiratory allergy (Ford, 2005; McLachlan, 2007). Most of the prospective studies have reported that obesity is a risk factor for the development of asthma, and a significant association between body fat and asthma, mainly in women, has been found.

White adipose tissue is composed of many cell types, including adipocytes and macrophages. Adipocytes secrete a variety of protein signals, namely adipokines, including leptin and adiponectin. Leptin has an influence on the hypothalamus by inducing satiety and by increasing energy metabolism. The serum concentration of leptin is increased in obese subjects suggesting a leptin resistance mechanism in obesity. Moreover, leptin protects T-cells from apoptosis, regulates their proliferation and activation, recruits and activates monocytes and macrophages, and promotes angiogenesis (Beutner, 2006; Fantuzzi, 2005).

Recently, it has been reported that leptin exerts a key role in the control of the proliferation of regulatory T lymphocytes (Tregs) (De Rosa, 2007). Thus, it has been hypothesized that an increased serum concentration of leptin would decrease the proliferation of Tregs. In addition, exogenous leptin has been shown to modulate allergic airway responses in mice independently of obesity. Leptin infusion caused enhanced airway hyperresponsiveness and an increase in serum IgE after inhaled ovalbumin challenge (Shore, 2005). Moreover, it has been demonstrated that human eosinophils express leptin surface receptors *in vitro* and *in vivo*, and leptin delays apoptosis of mature eosinophils *in vitro* (Wong, 2007). The anti-apoptotic effects of leptin are concentration dependent and blocked by an anti-leptin receptor monoclonal antibody.

The efficacy of leptin in blocking eosinophil apoptosis is similar to that of GM-CSF. Leptin delays the cleavage of Bax, as well as the mitochondrial release of cytochrome c, a second mitochondria-derived activator of caspase, suggesting that it blocks proapoptotic pathways proximal to mitochondria in eosinophils. Leptin is therefore a survival cytokine for human eosinophils, this finding suggests potential pathologic relevance in allergic and parasitic diseases. In fact, eosinophil is a relevant marker of allergic inflammation. Therefore, if leptin promotes eosinophilic survival, it might be hypothesized that leptin contributes to amplify allergic inflammation.

Leptin could also stimulate the chemokinesis of eosinophils, and induce the release of inflammatory cytokines, such as IL-1 β and IL-6, and chemokines IL-8, growth-related oncogene- α and MCP-1 (Conus, 2005). In view of these findings and the elevated production of leptin in patients with allergic diseases such as atopic asthma and atopic dermatitis, leptin could play crucial immunopathophysiological roles in allergic inflammation by activation of eosinophils via differential intracellular signaling cascades (Conus, 2005).

However, there are conflicting findings in allergic rhinitis. In fact, a first study reported that serum leptin levels were found to be significantly higher in patients with allergic rhinitis in the symptomatic period than normal controls (Unal, 2006). A next study demonstrated that the serum levels of leptin on allergic rhinitis were not different than in controls (Erel, 2007). However, both studies did not consider the type of sensitization, the gender, and the age of patients, and the relationship between leptin and clinical parameters.

Consequently, a further study investigated these issues, it was conducted in patients with seasonal allergic rhinitis evaluated outside the pollen season (Ciprandi, 2009). After analyzing genders separately, female allergic patients showed significantly higher levels than normal females ($p = 0.031$), whereas the difference between allergic and normal males was not significant ($p = 0.9651$).

Consequently, it may be hypothesized that leptin might play a role in allergic rhinitis, but it remained to demonstrated its role in the inflammatory process occurring during the pollen season. Therefore, the aim of this study was to evaluate also a group of patients with SAR, observed during the pollen season, and investigate the leptin serum concentration in this population comparing with patients evaluated outside the season and normal subjects.

METHODS

Study Design

The study included 137 subjects; 62 symptomatic SAR patients evaluated in season, 41 SAR patients without symptoms as evaluated outside the pollen season, and 34 normal subjects. All subjects were consecutively evaluated. The skin prick test and blood sampling for assessing serum leptin levels were performed in all subjects, that is, both allergic patients and healthy subjects. The study was approved by the local Ethics Committee and performed with the written informed consent of all participants. Allergic rhinitis was diagnosed according to validated criteria (Bousquet, 2008). Briefly, the diagnosis of allergic rhinitis was made on the basis of a positive history of nasal symptoms (i.e. nasal itching, sneezing, watery rhinorrhea, and nasal obstruction) during the pollen season since at least 2 years and positive skin prick test for pollen allergens.

Subjects

The median age of allergic patients evaluated out season, 19 females (46%) and 22 males (54%), was 39 years (range 14–75). The median age of allergic patients evaluated in season, 38 females (61%) and 24 males (39%), was 34 years (range 15–70). The median age of healthy subjects, 18 females (53%) and 16 males (47%), was 43 years (range 24–66). Therefore, the three groups were homogeneous for these demographic parameters.

Normal subjects were healthy and did not suffer from any endocrine-metabolic or chronic inflammatory/autoimmune disorders as excluded by doctors. Allergic patients were monosensitized to pollens alone and did not suffer from other chronic inflammatory/autoimmune or endocrine-metabolic disorders. Allergic patients were treated only with antihistamines on demand during the

pollen season. To minimize possible confounding factors, such as diet and circadian rhythms, all subjects were drawn in the morning and fasting. Moreover, in fertile females blood was drawn during the follicular phase.

Skin Prick Test

This assay was performed in all subjects, according to the guidelines of the European Academy of Allergy and Clinical Immunology (Dreborg, 1989). The panel of allergens employed consisted of: house dust mites (*Dermatophagoides farinae* and *pteronyssinus*), cat, dog, grass mix, *Compositae* mix, *Parietaria officinalis*, birch, hazel, olive tree, *Alternaria tenuis*, *Cladosporium*, and *Aspergilli* mix (Stallergenes, Milan, Italy).

Serum Leptin Assay

The Human Leptin Immunoassay (R&D Systems) employs the quantitative sandwich enzyme immunoassay technique and was performed in duplicates according to the Manufacturer's Instructions and expressed as ng/mL. Serum samples reported for males were (Range 2.20–11.149 ng/mL) and females (Range 3.87–77.27 ng/mL).

Symptom Severity

Allergic symptoms were scored by the patients referring to the immediately previous pollen season. The questionnaire included a visual analogue scale (VAS) of nasal symptoms according to validated criteria (Bousquet, 2007).

Statistical Analysis and Data Definitions

Descriptive statistics were first performed and quantitative parameters were reported as medians and standard deviation with first and third quartiles [interquartile range] due to the skewed distribution. The non-parametric Wilcoxon test was performed to evaluate the leptin differences between males and females, for allergic/non-allergic subjects. Correlation between quantitative variables was evaluated by means of the Pearson's correlation coefficient (r). All tests were two-sided and a p -value less than 0.05 was considered statistically significant. The package "S-Plus" (MathSoft Corp) was used for all the analyses.

RESULTS

Globally, the mean value of leptin in patients evaluate in season was 16.98 ng/ml, 8.61 ng/ml in patients evaluated outside the pollen season, and 7.09 in normal controls. The comparison between patients outside season and normal

controls was not significant ($p = 0.32$), whereas there was significant difference between patients evaluated outside season and patients evaluated in season ($p = 0.0002$) and between normal controls and patients evaluated in season. However, it is more appropriate stratifying patients for gender. Table 1 reports the serum levels in the three groups.

Differences between allergic males evaluated in season (10.78 ng/ml) and out of season (4.62 ng/ml) were significant ($p = 0.0004$); differences between allergic males evaluated in season and healthy males (4.05 ng/ml) were significant ($p = 0.0031$), but differences between allergic males studied out season and healthy males were not significant ($p = 0.9651$) (Figure 1a). Differences between allergic females evaluated in season (25.35 ng/ml) and out season (13.58 ng/ml) were not significant ($p = 0.0634$), whereas differences between allergic females evaluated in season and out of season and normal females (9.73 ng/ml) were significant ($p = 0.0002$ and $p = 0.031$, respectively): allergic females had higher levels than normal females (Figure 1b).

There was no significant relationship between serum levels and symptom severity in SAR patients evaluated during the pollen season (data not shown). There was a significant relationship between serum leptin and age only in females: allergic outside the pollen season ($r = 0.67$, $p < 0.004$) and controls ($r = 0.66$, $p < 0.005$).

DISCUSSION

There is evidence that obesity and weight gain are linked with allergic diseases probably through the immunological effects exerted by adipose tissue on the development of allergies (Beuther, 2006; Hersoug, 2007, Matricardi, 2007, McLachlan, 2007).

Allergic rhinitis is characterized by a Th2-polarized inflammation, which is closely dependent on allergen exposure (Ciprandi, 1995). This concept is very relevant as the presence of inflammation persists until allergen exposure occurs even though there are no symptoms: the so-called *minimal persistent inflammation*, phenomenon evidenced both in PAR and in SAR patients (Ciprandi, 1995; Ricca, 2000).

Leptin may be considered a pro-inflammatory cytokine as it is involved in eosinophil recruitment and it has been reported that serum leptin has increased in patients with acute allergic rhinitis (Unal, 2006). A further study provided evidence that SAR females had increased leptin production even though evaluated outside the pollen season, i.e., without inflammation and symptoms (Ciprandi, 2009). However, the effect of pollen exposure had to be demonstrated. Therefore, the present study was designed to confirm the possible role for leptin in SAR patients evaluated during the pollen season.

As previously reported (Ciprandi, 2009), we also felt that it was important to consider leptin levels separately for each gender. The first finding indicated

Table 1: Serum leptin levels expressed in ng/mL in males and females in the studied groups (number of subjects).

	Males			Females		
	In Season (24)	Out Season (22)	Healthy (16)	In Season (38)	Out Season (19)	Healthy (18)
25 th	7.50	1.36	1.55	14.44	8.73	6.09
Median	10.78	4.62	4.05	25.35	13.58	9.73
75 th	18.05	8.99	10.96	55.87	26.06	15.33
Standard Deviation	10.11	6.71	5.56	26.82	14.58	6.45

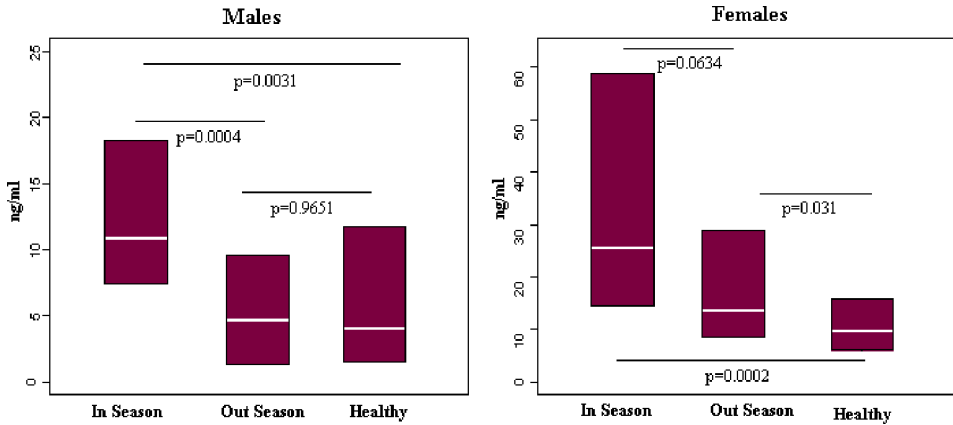


Figure 1: Leptin (ng/ml) distribution in allergic patients, evaluated in and out pollen season, and in healthy subjects, evaluated separately for males (a) and females (b). Values are represented as medians (white line), quartiles (25th and 75th percentiles, black box), and p-values between the groups.

that, though there was no significant difference in serum leptin between allergic males studied out season and normal males, in season allergic males had significantly higher levels than both out season patients and normal subjects as reported.

Moreover, we observed a significant difference between allergic out season females and normal females, this difference was more evident comparing allergic in season patients both with out season ones and normal subjects. However, there was no relationship between leptin levels and symptom severity. This finding might outline a pro-inflammatory effect exerted by leptin that might act on mechanisms not directly involved in symptom occurrence.

However, a very recent study showed no association between leptin plasma levels and rhinoconjunctivitis in children (Nagel, 2008). These conflicting results might be explained by the different characteristics of examined populations, mainly concerning the age, type of causal pollens (unfortunately the pediatric study did not provide specific IgE evaluation), clinical diagnosis, and duration of rhinitis. In this regard, it has been recently demonstrated that the duration of allergic rhinitis may have relevant clinical and functional consequences (Ciprandi 2008) that might depend on chronic stimulation of immune response. Moreover, the gender sub-analysis was not considered in this pediatric study.

In conclusion, this study has to be considered preliminary as shows some limitations: the lack of an homogeneous group evaluated both during and outside the pollen season, and the number of patients and controls might be higher. In addition, this investigation was a cross-sectional study conducted on one center, adjustment for potential confounder were not considered, and

the analysis was not adjusted for body mass index. Therefore, these findings should be considered preliminary. However, this study provides the evidence that serum leptin may depend on allergen exposure in SAR patients and provides support for the hypothesis that leptin may exert pro-inflammatory effects in this context.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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