Ghrelin and leptin levels in obese adolescents. 
Relationship with body fat and insulin resistance

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ABSTRACT
OBJECTIVE: Ghrelin and leptin levels are influenced by body fat (BF%), pubertal stage and possibly insulin resistance (IR). The aim of our study was: 1) To compare fasting ghrelin and leptin levels between obese and non-obese, adolescents, 2) to investigate possible correlations of these hormones with BF %, as well as IR. DESIGN: Twenty obese insulin resistant (IR) adolescents, twenty obese non IR (NIR) and fifteen healthy non-obese, age-matched adolescents were studied. In all participants, height, weight, body mass index (BMI) and BF % were measured. Fasting glucose, insulin, ghrelin and leptin levels were determined. IR was assessed using HOMA-IR index. RESULTS: BMI, BF %, insulin and HOMA-IR values were positively correlated with leptin and negatively with ghrelin levels. A negative correlation between circulating leptin and ghrelin levels was found. A suggestive positive correlation between leptin levels and BF %, independent of BMI, was also observed (P=0.075). Ghrelin levels were significantly correlated with insulin levels and HOMA-IR, independent of BMI (P=0.077). CONCLUSIONS: Obesity and IR may play an important role in the release of ghrelin as well as in the negative correlation between ghrelin and leptin.

Key words: Adolescents, Body fat, Ghrelin, Insulin resistance, Leptin, Obesity.

1. INTRODUCTION

Ghrelin and leptin are two hormones playing an important role in the regulation of food intake and body weight.1 Leptin is a 167 amino-residue peptide encoded by the obesity gene.2,3 It is secreted by fat tissue and suppresses food intake, while increasing energy expenditure.4 Following release into the circulation, leptin crosses the blood-brain barrier and binds to leptin receptors in the hypothalamus, influencing the activity of various hypothalamic neurons as well as the expression of various genes, encoding orexigenic and anorexigenic neuropeptides.5,6 Leptin levels are influenced by the amount of body fat, as they are found high in obese and low in lean individuals.7
Ghrelin is a 28 amino-residue peptide, produced predominantly by the stomach, and was initially discovered as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Among several other biological functions, ghrelin was found to produce positive energy balance by promoting food intake and decreasing energy expenditure in rodents. It plays a role in the regulation of energy balance and attenuates leptin-induced reduction in food intake and body weight. Ghrelin also modulates the expression of orexigenic and anorexigenic neuropeptides. Ghrelin levels were found decreased in obese individuals, with the exception of patients with Prader-Willi syndrome, and were found elevated in anorexia nervosa.

Ghrelin and leptin levels are also influenced by the pubertal stage. Leptin concentrations vary with Tanner stage independent of adiposity. They are higher in females than males at Tanner stages IV and V, but not at earlier stages of pubertal development. Ghrelin levels vary from fetal life through early adulthood. The highest levels of ghrelin are found during early postnatal life, when growth hormone begins to exert its effects on growth and important changes in food intake occur, suggesting that this hormone may participate in these processes. Prepubertal children have higher ghrelin concentrations than those in puberty.

Based on the above data, the present study was designed: 1) to compare fasting ghrelin and leptin levels between obese and non-obese adolescents, and 2) to investigate any correlation between these two hormones and body fat (BF %) as well as insulin resistance (IR).

**PATIENTS AND METHODS**

**Patients**

Twenty obese, insulin resistant (IR) adolescents (13 females, 7 males), aged (mean±SE) 12.86±1.82 years and twenty obese non insulin resistant (NIR) adolescents (10 females, 10 males), aged 12.72±1.87 years, with a mean body mass index (BMI) of 30.74±4.53 and 29.43±3.87, respectively (>97th percentile for age and sex), were included in the study. Insulin resistance (IR) was defined by the Homeostasis Model for the Assessment of Insulin Resistance (HOMA-IR) as a value >3.16 (mean±SE: 4.54±1.32).

Fifteen healthy non obese adolescents, matched for age, (9 females and 6 males, mean age 12.81±1.11 years), served as the control group (Table 1).

All the participants were screened by medical history and physical examination. None of them had any chronic disease or was undergoing any pharmacological treatment. By applying the five-stage system described by Tanner and Marshall to assess normal pubertal development, all were found to be pubertal. Body weight and height were measured in duplicate using a SEGA weighing-scale (SEGA 711) and stadiometer (HARPENDEN). Body mass index

| Table 1. Anthropometric, hormonal and metabolic features of the adolescents studied: mean ± SD, (range). x² or Kruskal-Wallis P values. |
|------------------|------------------|------------------|------------------|
|                  | OBESE ADOLESCENTS | CONTROLS (n=15)  | P                |
| Gender (M/F)     | IR (n=20)         | NIR (n=20)       |                  |
| Age (years)      | 12.86±1.82 (10.58-15.83) | 12.72±1.87 (10.83-16.00) | 12.81±1.11 (11.33-15.08) | =0.185 |
| BMI (kg/m²)      | 30.54±4.73 (23.63-46.00) | 29.43±3.87 (21.57-34.52) | 19.97±3.14 (15.90-25.80) | <0.001 |
| BF %             | 35.58±6.00 (25.20-47.90) | 34.33±5.14 (25.10-42.50) | 21.10±7.31 (9.80-32.70) | <0.001 |
| Glucose (mg/dl)  | 4.76±0.54 (4.03-5.70) | 4.77±0.59 (3.38-5.90) | 4.86±5.72 (3.93-5.58) | <0.867 |
| Insulin (μIU/ml) | 143.78±41.32 (89.68-246.82) | 134.64±19.01 (23.46-97.58) | 59.98±18.72 (30.06-92.55) | <0.001 |
| HOMA-IR          | 4.54±1.32 (3.16-8.49) | 1.80±0.55 (0.72-2.82) | 1.79±0.54 (0.98-2.90) | <0.001 |
| Leptin (ng/ml)   | 46.33±28.02 (10.70-121.10) | 34.74±17.93 (13.71-86.30) | 11.64±6.57 (2.10-24.29) | <0.001 |
| Ghrelin (pmol/l) | 860.12±289.89 (736.77-1619.36) | 1102.09±366.10 (735.47-1955.76) | 1358.47±401.15 (1011.87-2665.12) | <0.001 |

IR: insulin-resistant, NIR: non insulin-resistant; P<0.05 vs. controls. *P<0.05 vs. NIR obese adolescents.
(BMI) was calculated (weight in kilograms divided by the square of height in meters). Bioelectrical Impedance Analysis (BIA) (MALTRON analyzer BF-906) was applied to estimate body fat % (BF %), according to the manufacturer’s and NIH instructions.25

**Study protocol**

After an overnight fast, morning blood samples were drawn from all subjects included in the study. Blood samples were centrifuged (10 min at 1500g) and the aliquots were stored at –20°C until assayed according to the instructions published by Groeschl et al regarding ghrelin.26

In all groups, fasting glucose, ghrelin, leptin and insulin levels were measured. The obese adolescents underwent a standard oral glucose tolerance test (OGTT) – 1.75 g/kg body weight (max. 75g) of glucose per os—in order to exclude any participants having impaired glucose test (IGT) and to enable the use of fasting index HOMA-IR to define IR.27

The study was approved by the Scientific Ethics Committee of the Medical School of the Aristotle University of Thessaloniki. Informed written consent was obtained from the parents of all adolescents who underwent clinical and biochemical investigation.

**Assays and calculations**

**Glucose**

Serum glucose was determined with the glucose hexokinase enzymatic method (Architect c8000, Abbott Laboratories, Il, USA).

**Insulin**

Serum insulin was determined applying a solid-phase, two-site immunometric assay (Immulite 2000; DPC, USA), which had a coefficient of variation (CV) of 4.1-7.3% and a sensitivity of 2 µIU/ml.

**Leptin**

Serum leptin was determined by a commercial radioimmunoassay (DSL-23100 Active Human Leptin IRMA; DSL, USA). The lower limits of detection for leptin were 0.10 ng/ml, while inter-assay and intra-assay CV were 7.6 and 5%, respectively.

**Ghrelin**

Serum ghrelin was measured by radioimmunoassay (Phoenix Pharmaceuticals Inc., Belmont, CA, USA). This assay uses a 125I-labeled ghrelin tracer and a rabbit polyclonal antibody against full-length, octanoylated human ghrelin that recognizes the acylated and des-acyl forms. The antiserum does not cross-react with any relevant peptide, according to the information provided by the manufacturer. The lower and upper detection limits were 80 and 2500 pg/ml. The intra- and the inter-assay coefficients of variation (CV) were 5.30% and 13.61%, respectively.

**Insulin resistance**

Insulin resistance (IR) was assessed using the Homeostasis model assessment (HOMA-IR) according to the formula: fasting insulin (µIU/ml) x fasting glucose (mmol/l)/22.5.21

**Statistical analysis**

All analyses were performed by the statistical package SPSS, v.13.0 (SPSS Inc., Chicago, IL, USA). Two-tailed significance was set at 5%. The x² criterion was used to test for significant differences in gender distribution between the three groups. Given the relatively small number of cases per group, means were subsequently compared with the non-parametric Kruskal-Wallis test; pairwise comparisons were performed with the Mann-Whitney U-test, in cases of K-W P<0.05. Bivariate correlations were assessed with the Spearman coefficient. Independent correlations were evaluated by means of stepwise multiple regression and partial correlation analysis.

**RESULTS**

The anthropometric, hormonal and metabolic features of all adolescents studied are presented in Table 1. No statistically significant difference in age or gender distribution was observed between the three groups. No significant difference in ghrelin or leptin levels was observed between males and females in the two groups of obese adolescents (P=0.166 and 0.630, respectively). In the control group, females had significantly higher leptin levels (16.37±5.86) compared to males (8.43±5.07, P=0.022) (Table 2).

Obese adolescents (both IR and NIR) had significantly higher BMI and BF% values compared to controls (P<0.001 in all comparisons); no significant differences in BMI or BF% was observed between the two groups (IR and NIR) of obese adolescents.
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Table 2. Ghrelin and leptin levels in male (M) and female (F) adolescents studied separately (mean ± SD, range, Mann-Whitney P values).

<table>
<thead>
<tr>
<th></th>
<th>Obese Adolescents IR</th>
<th>Obese Adolescents NIR</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>M</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>44.18±20.06</td>
<td>47.26±31.46</td>
<td>=0.444</td>
</tr>
<tr>
<td>F</td>
<td>17.12-66.63</td>
<td>10.70-121.10</td>
<td></td>
</tr>
<tr>
<td>Ghrelin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>903.44±290.28</td>
<td>841.55±298.63</td>
<td>=0.968</td>
</tr>
<tr>
<td>F</td>
<td>419.93-1285.20</td>
<td>376.77-1619.36</td>
<td></td>
</tr>
</tbody>
</table>

IR: insulin-resistant, NIR: non-insulin-resistant, P values <0.05 are significant.

Notably, a suggestive positive correlation between leptin concentrations and BF%, independent of BMI, was also observed (P=0.072) (Table 3).

Ghrelin levels were significantly negatively correlated with fasting insulin levels and HOMA-IR values (P=0.022) (Table 3, Figure 3), independent of BMI (Table 3).

In multiple regression analysis, leptin levels were independently associated only with BMI values (B=2.55±4.32, R²=3.98; P<0.001). By contrast, one

![Figure 1. Serum leptin levels in obese insulin-resistant (OBESE IR) adolescents, obese non-insulin-resistant (OBESE NIR) adolescents and lean adolescents (CONTROLS). Box-plots extend from the 25th to the 75th percentile and whiskers to the largest and smallest observed values within 1.5 box lengths; the solid line is the median.](image-url)
and probably in the interaction between ghrelin and leptin. In the present study, this relationship was substantiated by the significant negative correlation of markers of IR with ghrelin levels, independent of obesity (Table 3, Figures 2 and 3).

Regarding the exact relationship between ghrelin and insulin, conflicting results have been reported. In a study conducted by Adeghate et al, ghrelin was found to stimulate insulin secretion from the pancreas of normal and diabetic rats, whereas Egido et al reported an inhibitory effect of ghrelin on insulin and somatostatin secretion. Schaller et al, on the other hand, reported that plasma ghrelin concentrations are not regulated by glucose or insulin. According to this study, hyperinsulinemia at concentrations typically seen in IR did not affect plasma ghrelin levels. In the same study, it was observed that insulin at pharmacological concentrations caused a dose-dependent decrease in circulating plasma ghrelin. Glucose in combination with supraphysiological insulin concentrations might cross the blood-brain barrier more easily and influence the central regulation of gastric ghrelin release. Saad et al reported that insulinemia possibly mediates the effect of nutritional status and energy balance on plasma ghrelin. Notably, insulin could play a pivotal role in regulating body weight through its down-regulating effects on plasma ghrelin concentrations. Finally, Erdman et al have reached the conclusion that in obese subjects with associated hyperinsulinemia, ghrelin suppression is due to insulin, whereas leptin can be important for reduction of ghrelin release during moderate increases of body weight.

The BMI-independent relationship between leptin

<table>
<thead>
<tr>
<th>LEPTIN</th>
<th>Bivariante</th>
<th>Partial for BMI</th>
<th>GHRELIN</th>
<th>Bivariante</th>
<th>Partial for BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r_spearman</td>
<td>r_p_corr</td>
<td></td>
<td>r_spearman</td>
<td>r_p_corr</td>
</tr>
<tr>
<td>BMI</td>
<td>+0.735</td>
<td>&lt;0.001</td>
<td>---------</td>
<td>-0.490</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BF%</td>
<td>+0.617</td>
<td>&lt;0.001</td>
<td>+0.247</td>
<td>=0.072</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>+0.497</td>
<td>&lt;0.001</td>
<td>+0.244</td>
<td>=0.075</td>
<td>-0.497</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>+0.502</td>
<td>&lt;0.001</td>
<td>+0.242</td>
<td>=0.077</td>
<td>-0.454</td>
</tr>
<tr>
<td>Leptin</td>
<td>---------</td>
<td></td>
<td>---------</td>
<td>-0.466</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>-0.466</td>
<td>&lt;0.001</td>
<td>-0.111</td>
<td>=0.425</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Bivariate and independent (partial), controlling for BMI, correlations of serum leptin and ghrelin levels to parameters of obesity and insulin resistance in ll adolescents studied.
In the literature it is well documented that leptin has gender differences. Although prepubertally leptin levels do not differ between boys and girls, with the onset of puberty leptin levels increase in girls and decrease in boys. This could be explained by the fact that girls gain more fat mass than boys, whereas boys gain more fat-free mass. This marked sexual dimorphism that is present in serum leptin levels at puberty, especially when weight is considered, result from genotype by sex interactions mediated by testosterone. Genes which influence variation in leptin levels are differentially expressed depending on sex and, moreover, sexes show differences in response to the expression of this obesity-related trait to unmeasured residual effects. This sexual dimorphism of leptin was confirmed in our study only in the group of normal-weight individuals (control group), in which females had significantly higher leptin levels compared to males. This is in agreement with the study conducted by Nakanishi et al in which sexual dimorphism in leptin levels and relative weight was found in normal-weight but not in overweight children and adolescents.

There are conflicting data with regard to gender differences in ghrelin levels. In certain studies, ghrelin levels were found comparable in men and women, while in others the observed differences in ghrelin levels according to gender refer only to healthy individuals. Thus in the study conducted by Makovey et al in the obese group no significant gender differences were found. In our study, the absence of statistical significance in ghrelin levels between male and female adolescents could be attributed to the relatively small number of individuals involved.

It is known that ghrelin and leptin are two secreted peptides which apparently play an important role in the regulation of food intake and body weight. Leptin, acts on regulatory centres in the brain to inhibit food intake and increase energy expenditure, thus acting as a long-term regulator of body weight. Ghrelin, on the other hand, is a fast-acting hormone that operates as a meal-initiation signal in the system for short-term regulation of energy balance. Therefore, it is possible that a negative interplay between the two hormones exists, as demonstrated in the present
study (Figure 4).

In rodents, leptin has been shown to be an upstream regulator of ghrelin, whereas in humans, several studies have produced conflicting results. Tschöp et al demonstrated that, in obese individuals, fasting leptin levels are negatively correlated with fasting ghrelin concentrations. However, in a study conducted by Ikezaki et al in obese children and adolescents, fasting leptin and ghrelin levels were not significantly correlated. As mentioned above, the results of the present study support the notion that leptin and ghrelin levels are negatively correlated (Figure 4).

Regarding the negative association between ghrelin and leptin that was found in our study, this could simply reflect BMI associations. An inverse relationship between leptin and ghrelin has been reported in several studies which would support a contribution of leptin to obesity-related low ghrelin levels. However, the experimental evidence for such a negative feedback control is still a matter of debate. In mice, leptin administered intraperitoneally inhibits ghrelin release, while in rats leptin can prevent the rise of ghrelin during food restriction. In contrast to these findings in rodents, Chan et al reported that administration of recombinant leptin in lean individuals had no effect on plasma ghrelin concentrations. It is speculated that through the hypothalamic/pituitary axis ghrelin and leptin operate as a metabolic switch.

Ghrelin suppression may be another feature of IR in obese adolescents. It should be noted that the estimation of IR by means of fasting indices, instead of the golden standard (i.e. the hyperinsulinemic euglycemic clamp), poses some limitations in the interpretation of our results. Other limitations include the relatively small number of subjects and the use of BIA for the estimation of BF %.

In conclusion, the findings of the present study support the thesis that ghrelin is negatively correlated with leptin, obesity and insulin resistance. In adolescents, ghrelin is negatively correlated with IR, independent of obesity. The exact relationship between leptin, ghrelin, body fat and insulin resistance needs to be further clarified by both in vitro experiments and larger-scale studies.

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