**Abstract.** – **Objectives:** The insulin-mimetic adipocytokine visfatin has been related to obesity. The aim of this study was to examine whether weight loss could change visfatin concentrations in morbidly obese patients and its relationship with insulin resistance.

**Material and Methods:** This was an interventional study analyzing a population of 41 morbidly obese patients. A biochemical analysis was realized before and after 2 months on a hypocaloric diet.

**Results:** After weight loss (average 4.41%), BMI, weight, fat mass, fat free mass, waist circumference, systolic blood pressure, serum glucose, total cholesterol, insulin and HOMA decreased. The serum concentrations of visfatin did not decrease (43.5+30.8 vs. 47.1+38.1 ng/ml). In the multivariate analysis visfatin concentrations as a dependent variable, only C reactive protein remained as an independent predictor in the model before diet, with an increase of 1.82 ng/ml (CI95%:0.02-3.61) basal visfatin concentrations with each increase of 1 mg/dl of CRP. Only HOMA remained as an independent predictor in the model after diet, with an increase of 11.4 ng/ml (CI95%:1.76-21.11) post-treatment visfatin concentrations with each increase of 1 unit HOMA.

**Conclusion:** Weight reduction after a 2 months on a hypocaloric diet is not associated with a significant change in circulating visfatin in morbidly obese patients.

**Key Words:** Adipocytokine, Hypocaloric diet, Morbid obesity, Visfatin.

**Introduction**

Obesity is associated with cardiovascular risk factors, including altered levels of inflammatory markers and adipocytokines and may be regarded as a low-grade inflammatory state. Epidemiologic evidence of this rising tide of obesity and associated pathologies has led, in the last years, to a dramatic increase of researches on the role of adipose tissue as an active participant in controlling pathologic processes. The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate cardiovascular risk factors and inflammation. The association between accumulation of visceral adipose tissue and insulin resistance is well established in obesity and type 2 diabetes, and both visceral fat and insulin resistance are associated with increased cardiovascular risk. Dieting or surgically induced weight loss may reduce this inflammatory state and adipocytokine levels.

Visfatin was recently identified as a protein preferentially expressed in visceral adipose tissue. Interestingly, visfatin expression is regulated by cytokines that promote insulin resistance, such as TNF alpha and IL-6. Visfatin has an endocrine role and it might also have a paracrine effect on the visceral adipose tissue through its pro-adipogenic and lipogenic actions. Visfatin exhibit insulin mimetic properties resulting in a glucose lowering effect. The discovery of this curious new adipokine has great potential to significantly enhance our understanding of the metabolic syndrome and its treatment.

Morbid obesity may be a perfect model to study the role and the response of visfatin to a weight loss program. Little evidence has been published in interventional studies. The aim of our study was to examine the changes on visfatin levels after weight reduction in morbidly obese patients and its relationship with insulin resistance.
Subjects and Methods

Subjects
A population of 41 morbidly obese patients (body mass index >40) was enrolled. These patients were studied in a Nutrition Clinic Unit. Ethics Committee approved all experimental procedures in accordance with Helsinki Declaration. Exclusion criteria included active infectious disease, history of cardiovascular disease or stroke during the previous 36 months, total cholesterol > 300 mg/dl, triglycerides > 400 mg/dl, blood pressure > 145/90 mmHg, diabetes mellitus >126 mg/dl, as well as the use of glucocorticoids, angiotensin receptor blockers, angiotensin converting enzyme inhibitors and spironolactone within the previous 3 months.

Procedure
A hypocaloric 1520 kcal diet containing 52% of carbohydrates, 25% of lipids and 23% of proteins was administered for a 2 months period. A dietitian supervised the weight loss and diet compliance with written food records. Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, visfatin and insulin resistance (HOMA, Homeostatic model assessment) levels were measured.

Assays
Serum glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured by enzymatic colorimetry (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Serum visfatin was analyzed using a commercially available ELISA kit (Phoenix Peptides, Belmont, CA, USA). Assay sensitivity was 2 ng/ml and interassay and intraassay coefficients of variation were less than 10% and less than 5%, respectively.

Indirect Calorimetry
For the measurement of resting energy expenditure, subjects were admitted to a metabolic ward. After a 12 h overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20 min period with an open-circuit indirect calorimetry system (standardized for temperature, pressure and moisture) fitted with a face mask (MedGem;Health Tech, Golden, CO, USA), coefficient of variation 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated.

Anthropometric and Blood Pressures Measurements
Body weight was measured to an accuracy of 0.1 Kg and body mass index computed as body weight/(height²). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition. Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygomanometer, and averaged.

Dietary Intake
All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was made through computer equipped and personal software, with food scales and models, to enhance portion size accuracy. Records were reviewed by a dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference.

Statistical Analysis
Sample size was calculated to detect differences of 25% on HOMA after diet treatment with 80% power and 5% significance. The results were expressed as mean ± standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student’s-t test. Non-parametric variables were analyzed with the U Mann Whitney. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. A multivariate regression models (enter mode) were used to study
the dependent variables (basal visfatin, post-treatment visfatin and visfatin changes) adjusted by age and sex. A p-value under 0.05 was considered statistically significant.

Results

Forty one patients were invited to participate in the present study. The mean age was 49.9±15.7 years, the mean BMI was 44.6±5.6 with 8 males (19.5%) and 33 females (80.5%).

Basal assessment of nutritional intake (pre-treatment) with 3-d written food records showed a mean caloric intake of 1741.9±812.7 kcal/day, a carbohydrate intake of 180.7±51.3 g/day (41.9% of calories), a fat intake of 74.1±30.4 g/day (38.3% of calories) and a protein intake of 84.9±25.5 g/day (19.8% of calories). These patients reached the recommendations of diet (1428 kcal/day, with a 49% of carbohydrates, 28% of lipids and 23% of proteins).

Table I shows the differences in anthropometric variables. All patients lost weight and less than 5% (average 4.41%). After weight loss, BMI, weight, fat mass, fat free mass, waist circumference and systolic blood pressure decreased.

Table II shows the differences in cardiovascular risk factors. After weight loss, a decrease in serum glucose, total cholesterol, insulin and HOMA was detected.

Serum visfatin concentrations did not decrease in (43.5±30.8 vs. 47.1±38.1 ng/ml). Sex differences were analyzed in visfatin response. Basal levels of visfatin were similar in males and females (33.4±30.8 vs 40.1±10.7 ng/ml).

In pre-treatment correlation analysis, basal visfatin concentrations showed a significant association with CRP (r=0.47; p=0.005). After weight loss, visfatin concentrations showed a significant association with HOMA (r=0.53; p=0.04) and glucose concentrations (r=0.61; p=0.01).

In the multivariate analysis with age- and sex-adjusted basal visfatin concentrations as a dependent variable, only C reactive protein remained as an independent predictor in the model (F=4.5; p<0.05), with an increase of 1.82 ng/ml (CI95%: 0.02-3.61) basal visfatin concentrations with each increase of 1 mg/dl of CRP. In the second multivariate analysis with age- and sex-adjusted basal post treatment visfatin concentrations as a dependent variable, only HOMA remained as an independent predictor in the model (F=5.2; p<0.05), with an increase of 11.4 ng/ml (CI95%: 1.76-21.11) post-treatment visfatin concentrations with each increase of 1 unit HOMA.

Discussion

The finding of this study was that a moderate weight reduction after a hypocaloric diet in morbidly obese patients is not associated with a significant change in circulating concentrations of visfatin. Moreover, before to the diet, visfatin levels were associated with C reactive protein levels and after the hypocaloric diet with HOMA.

Table I. Changes in anthropometric variables.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Baseline</th>
<th>2 months</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>44.6 ± 5.5</td>
<td>42.8 ± 5.4*</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>113.1 ± 18.9</td>
<td>108.5 ± 18.1*</td>
<td>0.001</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>55.9 ± 17.1</td>
<td>54.1 ± 16.5*</td>
<td>0.004</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>53.1 ± 13.7</td>
<td>51.9 ± 11.1*</td>
<td>0.005</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>126.2 ± 13.0</td>
<td>122.6 ± 12.8*</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.94 ± 0.08</td>
<td>0.93 ± 0.09</td>
<td>0.370</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>141.8 ± 19.5</td>
<td>123.7 ± 29.5*</td>
<td>0.040</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>81.2 ± 11.3</td>
<td>83.8 ± 6.6</td>
<td>0.670</td>
</tr>
<tr>
<td>RMR(kcal/day)</td>
<td>2112 ± 454</td>
<td>2173 ± 568</td>
<td>0.652</td>
</tr>
<tr>
<td>VO2c. (ml/min.)</td>
<td>281.6 ± 87.4</td>
<td>307.7 ± 89.3</td>
<td>0.673</td>
</tr>
</tbody>
</table>

RMR: resting metabolic rate. O2c.: Oxygen consumption. BP: Blood pressure. t Student test and Wilcoxon test were used as statistical methods. (*) p<0.05, in each group with basal values.
Visfatin was reported to be more highly expressed in visceral than subcutaneous fat, and visceral fat may be increased in insulin-resistant patients\textsuperscript{13}. Our results did not show a correlation between visfatin concentrations and anthropometric parameters, such as waist circumference, fat mass or lean mass, as shown by previous studies\textsuperscript{13}.

In morbidly obese patients, our results showed a correlation with HOMA and serum glucose concentrations. In contrast Li et al\textsuperscript{14} have demonstrated that visfatin concentrations were significantly decreased in diabetics compared with the controls and were correlated positively and significantly with BMI, waist to hip ratio and resistin. However, Dogru et al\textsuperscript{15} have demonstrated that visfatin levels did not correlate with BMI or HOMA in three groups of subjects (type 2 diabetes mellitus, impaired glucose tolerance and controls).

Studies investigating the molecular mechanisms revealed that visfatin activates the intracellular signaling cascade for insulin. Interestingly, visfatin activates the insulin receptor in a manner distinct from that of insulin. Characterizing this activation step will be an important challenge, because this may identify novel targets for the development of antidiabetic therapy. However, treatment with thiazolidinediones for 3 weeks did not increase mRNA or circulating visfatin levels in either group\textsuperscript{10}.

In our study, parameters related with metabolic syndrome clearly decreased after weight loss on these patients. Visfatin is mainly secreted from visceral fat. A decreased amount of visceral fat (decrease of waist circumference) would suggest a reduction in visfatin concentrations after hypocaloric diet. This data is according to findings in a recent report\textsuperscript{14}. However, in other study, weight loss after gastric surgery was associated with an increase in visfatin\textsuperscript{15}.

Few studies have been designed to explore changes on visfatin levels after weight loss. In morbidly obese patients, elevated visfatin concentrations were reduced after weight loss\textsuperscript{7}. This study has some methodological differences with ours; patients were treated with gastric banding and followed up 6 months. The weight loss was over 16 kg. Recently, Manco et al\textsuperscript{8} have reported, that surgically weight loss reduced slightly visfatin concentrations in the limit of the significance ($p<0.05$). This study has some differences with as, too; patients were treated with biliopancreatic diversion, and follow up 36 months. Weight loss was over 29 kg. In other previous study\textsuperscript{6}, moderate weight loss (3 kg) after 3 months on a hypocaloric diet was associated with a significant decrease in circulating concentrations of visfatin.

On the other hand, plasma levels of visfatin in severely obese patients (averaged BMI over 54) were increased after intestinal bypass\textsuperscript{16}. Krzyzanowska et al\textsuperscript{17} reported that massive weight loss after gastroplasic surgery is accompanied by an increase in circulating concentrations of visfatin. Kovacikova et al\textsuperscript{18} have demonstrated an increase of visfatin mRNA in subcutaneous abdominal adipose tissue after weight reduction, the diet induced increase was positively correlated with the magnitude of body weight loss.

These contradictory findings in the literature on visfatin concentrations after weight loss could...
be explained by different inclusion criteria of subjects. Firstly, the average BMI was different in previous studies. Secondly, the amount of weight loss, the percentage of weight loss and the maintenance of this were different, too. Thirdly, the type of bariatric surgery (intestinal bypass, banding, gastroplastic and bilipancreatic diversion) and the type of hypocaloric diet could be the main factor. Macronutrients distribution of the diets rather than total calorie restriction could influence visfatin response, too.

Our results showed a positive correlation between visfatin and C-reactive protein in pre-treated patients and visfatin with HOMA in post-treated patients. The lack of association reported in previous studies between insulin resistance and visfatin might be explained by BMI differences, only it would be observed in morbidly obese patients with marker insulin resistance. Berndt et al reported a lack of relationship between visfatin and insulin sensitivity assessed by euglycemic hyperinsulinemic clamp. The connection between visfatin and insulin resistance is controversial and might differ according to patient cohorts and a pro-inflammatory status might be involved in this association as shown C reactive protein relationship.

Perhaps a secondary hypothesis could explain the unclear results of the literature. Haider et al have been detected that the release of visfatin by adipocytes was dependent on duration and magnitude of glucose elevations. These data suggest that visfatin release may represent a nutrient sensor of adipocytes and fail to detect a relationship between the metabolic syndrome and visfatin.

In conclusion, weight reduction after a 2 months on a hypocaloric diet is not associated with a significant change in circulating visfatin in morbidly obese patients. Visfatin is related to an inflammatory status and HOMA, too.

References


