Circulating adipocytokines in morbid obese patients, relation with cardiovascular risk factors and anthropometric parameters

D. A. De Luis, M. González Sagrado, R. Conde, R. Aller, O. Izaola and M.ª J. Castro


Abstract

Background: Obesity and insulin resistance are associated with cardiovascular risk factors, including adipocytokines. The aim of the present study was to explore the relation of circulating adipocytokines with cardiovascular risk and anthropometric parameters in morbid obese patients.

Subjects: A population of 65 morbid obese patients was analyzed in a prospective way. A biochemical, anthropometric and dietary evaluation was realized.

Results: In the multivariate analysis with resistin as dependent variable, the BMI remained in the model (F = 4.46; p < 0.05), with an increase of 0.43 (CI 95%: 0.10-0.76) pg/ml with each point of BMI. In the second model with adiponectin as dependent variable, the age remained in the model (F = 16.6; p < 0.05), with an increase of 0.04-0.51) pg/ml with each kg of weight. In the fourth model with TNF-alpha as dependent variable, resistin, IL-6 and weight remained in the model (F = 8.8; p < 0.01), with an increase of 0.26 (CI 95%: 0.05-0.47) pg/ml with each point of HOMA, an increase of 10.35 (CI 95%: 4.10-21.12) ng/ml with each point of BMI and a decrease of 10.16 (CI 95%: -20.37-0.76) pg/ml with each 1 pg/dl of TNF-alpha.

Conclusions: Circulating adipocytokine concentrations are associated with different cardiovascular risk factors and anthropometric variables in morbid obese patients.


Key words: Adipocytokines. Anthropometry. Cardiovascular risk factors. Morbid obesity.
Introduction

Obesity and insulin resistance are associated with cardiovascular risk factors, including altered levels of inflammatory markers and adipocytokines. This association is related with body mass index, patients with morbid obesity have high cardiovascular risk. Obesity is characterized by a low grade systemic inflammation. Epidemiological evidence of this rising tide of obesity and associated pathologies has led to a dramatic increase of research on the role of adipose tissue as an active participant in controlling the body's physiologic and pathologic processes.

The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate insulin sensitivity, energy expenditure and inflammation. Morbid obesity (body mass index > 40) could be used as a model to explain these interesting relationships due to the high percentage of fat mass.

Adipocytokines are proteins produced mainly by adipocytes. Adiponectin is an adipocyte-derived collagen like protein identified through an extensive search of adipose tissue. Hypoadiponectinemia increased risk of coronary artery disease together with the presence of multiple risk factors, indicating that adiponectin is a key factor of the metabolic syndrome. Leptin is a protein secreted primarily from adipocytes, too. Leptin suppresses food intake and increase energy expenditure by enhancing thermogenesis and metabolic rate. Recent reports suggest that leptin contributes to atherosclerosis and cardiovascular disease in obese patients. Resistin is a cysteine-rich protein identified by screening for the genes that are induced during the differentiation of the adipocytes. Although the role of resistin in linking human obesity with type 2 diabetes mellitus is thus far questionable, TNF alpha and interleukin 6 are increased in most animal and human models with obesity and insulin resistance. Recently, in a non morbid obese sample of patients have been demonstrated interesting relationships with adipocytokines.

The aim of the present study was to explore the relation of circulating adipocytokines with cardiovascular risk and anthropometric parameters in patients with morbid obesity.

Subjects and methods

Subjects

A population of 65 morbid obese (body mass index > 40) patients was analyzed in a cross sectional observational study. These patients were studied in a Nutrition Clinic Unit after and they gave written informed consent. All the procedures were approved by Ethics Committee according to Helsinki Declaration.

Procedure

All patients with a 2 weeks weight-stabilization period before recruitment were enrolled. Weight, blood pressure, basal glucose, lipoprotein (a), c-reactive protein (CRP), insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides blood and adipocytokines (leptin, adiponectin, resistin, Interleukin-6 (IL-6) and TNF alpha) levels were measured.

Assays

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 supernantant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). 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.

Adipocytokines

Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml. Adiponectin was measured by ELISA (R&D systems, Inc., Mineapolis, USA) with a sensitivity of 0.246 ng/ml and a normal range of 865-21424 ng/ml. Interleukin 6 and TNF alpha were measured by ELISA (R&D systems, Inc., Mineapolis, USA) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNFalpha (0.5-15.6 pg/ml).

Indirect calorimetry

In order to measure resting energy expenditure, subjects were admitted to a metabolic ward. After a 12-hour overnight fast, resting metabolic rate was measured in each subject, awake and seated, in a temperature-controlled room over one 20-minute period with an open-circuit indirect calorimetry system (standardized for temperature, pressure and moisture). The subject was fitted with a face mask (MedGem;Health Tech, Golden, USA). The coefficient of variation was 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated.
Anthropometric measurements and blood pressure

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. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass.

Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged.

Dietary intake

Patient’s nutritional intake was assessed prospectively by analysis of written food records. All subjects enrolled in the study were instructed to record their daily dietary intake for three days, including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analysed with a computer-based data evaluation system. National composition food tables were used as references. Regular aerobic physical activity (walking was allowed, no other exercises) was maintained during the period study (120-180 minutes at least 60% of maximal heart frequency).

Statistical analysis

The results were expressed as average 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 and ANOVA test. Non-parametric variables were analyzed with the Friedman and Wilcoxon tests. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. Correlation analysis was realized with Spearman and Pearson tests. A multiple regression model (step by step) was used to study the dependent variables (leptin, adiponectin, resistin, TNF alpha, and interleukin 6). A p-value under 0.05 was considered statistically significant.

Results

Sixty five patients gave informed consent and were enrolled in the study. The mean age was 48.2 ± 15.4 years, the mean BMI 44.4 ± 3.9 and the mean weight 114.7 ± 18.1. Sex baseline characteristics of patients are presented in table I, with higher HOMA and insulin levels in men than women and higher HDL cholesterol and lipoprotein (a) levels in women than men. All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.4 ± 0.3 kg). Anthropometric measurements showed an average waist circumference (125.6 ± 12.5 cm) and waist-to-hip ratio (0.94 ± 0.08). Tetrapolar body electrical bioimpedance showed the next data; fat free mass (55.7 ± 16.3 kg) and fat mass (56.7 ± 14.5 kg). Indirect calorimetry showed a resting metabolic rate (RMR) (2326.3 ± 653.4 kcal/day) and oxygen consumption (324.1 ± 87.2 ml/min). Table II shows differences between men and women, with higher weight, fat mass, fat free mass, waist circumference, waist to hip ratio, RMR and oxygen consumption in men than women.

Serial assessment of nutritional intake with 3 days written food records showed a calorie intake of 1,845 ± 624 kcal/day, a carbohydrate intake of 192.5 ± 73.68

<table>
<thead>
<tr>
<th>Table I</th>
<th>Clinical and biochemical characteristics of study population</th>
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<tbody>
<tr>
<td>Characteristics</td>
<td>Male (n = 12)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.0 ± 18.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>44.4 ± 3.3</td>
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<tr>
<td>Systolic BP (mmHg)</td>
<td>137.1 ± 11.6</td>
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<tr>
<td>Diastolic BP (mmHg)</td>
<td>88.3 ± 7.8</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>103.8 ± 16.4</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>196.7 ± 41.1</td>
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<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>116.1 ± 27.2</td>
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<tr>
<td>HDL-cholesterol (mg/dl)</td>
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</tr>
<tr>
<td>Lipoprotein(a) (mg/dl)</td>
<td>9.5 ± 7.1</td>
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<tr>
<td>Insulin (mUI/L)</td>
<td>40.7 ± 25.1</td>
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<tr>
<td>HOMA</td>
<td>10.9 ± 8.7</td>
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<tr>
<td>CRP (mg/dl)</td>
<td>6.56 ± 4.1</td>
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</table>

BP: Blood pressure. CRP: c reactive protein.

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<tr>
<th>Table II</th>
<th>Anthropometric characteristics by sex</th>
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<tbody>
<tr>
<td>Characteristics</td>
<td>Male (n = 12)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>129.9 ± 17.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.10</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>83.3 ± 7.8</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>45.8 ± 7.8</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>134.2 ± 14.9</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td>RMR (kcal/day)</td>
<td>2.475 ± 814</td>
</tr>
<tr>
<td>O₂ c. (ml/min.)</td>
<td>331.1 ± 115</td>
</tr>
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</table>

RMR: resting metabolic rate. O₂ c.: Oxygen consumption.
g/day, a fat intake of 78.4 ± 34.8 g/day and a protein intake of 89.4 ± 26.4 g/day. No significantly differences were detected in dietary intakes between males and females: calories (1,887.6 ± 593 kcal/day vs 1,835 ± 479 kcal/day, ns), carbohydrates (187.8 ± 46.6 g/day vs 193.6 ± 79.1 g/day, ns), proteins (93.0 ± 28.9 g/day vs 88.4 ± 26.2 g/day, ns) and lipids (81.2 ± 51 g/day vs 77.7 ± 33 g/day, ns).

Table III shows circulating levels of adipocytokines, leptin and adiponectin levels were higher in women than men.

Correlation analysis showed a significant correlation among leptin levels and the independent variables; diastolic blood pressure (r = -0.48, p = 0.002), resistin (r = -0.45, p = 0.01), TNF-alpha (r = -0.32, p = 0.03). Adiponectin levels were correlated with age (r = 0.61; p = 0.02), HOMA (r = -0.33; p = 0.03). Interleukin 6 were correlated with TNF-alpha (r = 0.57; p < 0.001), weight (r = 0.35; p = 0.01), BMI (r = 0.42; p = 0.002), systolic blood pressure (r = 0.28; p = 0.03), c-reactive protein (r = 0.39; p = 0.007). Resistin showed a correlation with BMI (r = 0.37; p = 0.01), systolic blood pressure (r = 0.35; p = 0.02), triglycerides (r = 0.30; p = 0.04), LDL-cholesterol (r = 0.35; p = 0.02), leptin (r = -0.45; p = 0.01), TNF-alpha (r = 0.39; p = 0.03), c-reactive protein (r = 0.35; p = 0.008). TNF-alpha was correlated with BMI (r = 0.35; p = 0.03), weight (r = 0.42; p = 0.004), resistin (r = 0.57; p = 0.001), diastolic blood pressure (r = 0.30; p = 0.04), leptin (r = -0.32; p = 0.03) and IL 6 (r = 0.39; p = 0.03).

In women, correlation analysis showed a significant correlation among leptin levels and the independent variables; resistin (r = -0.54; p = 0.01), TNF-alpha (r = -0.37; p = 0.03), diastolic blood pressure (r = -0.61; p = 0.01). Adiponectin levels were correlated with age (r = 0.53; p = 0.02) and c-reactive protein (r = -0.44; p = 0.02). Interleukin 6 were correlated with TNF-alpha (r = 0.54; p = 0.001), weight (r = 0.34; p = 0.01), BMI (r = 0.37; p = 0.02), systolic blood pressure (r = 0.33; p = 0.03), c-reactive protein (r = 0.39; p = 0.009). Resistin showed a correlation with leptin (r = -0.54; p = 0.01) and triglycerides (r = -0.31; p = 0.03). TNF-alpha was correlated with weight (r = 0.48; p = 0.03), resistin (r = 0.57; p = 0.001), diastolic blood pressure (r = 0.38; p = 0.02), leptin (r = -0.37; p = 0.03) and IL 6 (r = 0.54; p < 0.001).

In men, correlation analysis showed a significant correlation among leptin levels and the independent variables; resistin (r = -0.83; p = 0.001), triglycerides (r = -0.92; p = 0.02), systolic blood pressure (r = -0.69; p = 0.01) and C-reactive protein (r = -0.75; p = 0.03). Adiponectin levels were correlated with age (r = 0.63; p = 0.02) and HOMA (r = -0.77; p = 0.002). Interleukin 6 were correlated with glucose (r = 0.95; p = 0.001) and BMI (r = 0.64; p = 0.02). Resistin showed a correlation with leptin (r = -0.83; p = 0.001), systolic blood pressure (r = -0.63; p = 0.03), total cholesterol (r = 0.69; p = 0.013), c-reactive protein (r = 0.66; p = 0.02), LDL-cholesterol (r = 0.64; p = 0.02), and triglycerides (r = -0.31; p = 0.03). TNF-alpha was correlated with total cholesterol (r = 0.94; p = 0.03) and LDL-cholesterol (r = 0.93; p = 0.001).

After univariate analysis, we performed a multivariate analysis. In this analysis adjusted, by age and sex with a dependent variable (resistin), the BMI remained in the model (F = 16.6; p < 0.05), with an increase of 0.23 (CI 95%: 0.06-0.41) ng/ml with each point of BMI. In a second model adjusted by sex and fat mass with a dependent variable (adiponectin), the age remained in the model (F = 4.46; p < 0.05), with an increase of 3.62 (CI 95%: 0.05-7.21) ng/ml with each year. In the third multivariate analysis adjusted by age, sex and fat mass with a dependent variable (interleukin 6), the HOMA, CRP and weight remained in the model (F = 8.8; p < 0.01), with an increase of 0.26 (CI 95%: 0.05-0.47) pg/ml with each point of HOMA, an increase of 0.43 (CI 95%: 0.10-0.76) pg/ml with each 1 mg/dl of CRP and an increase of 0.13 (CI 95%: 0.05-0.21) pg/ml with each kg of weight. In the fourth multivariate analysis adjusted by age, sex and fat mass with a dependent variable (TNF-alpha), resistin, IL-6 and weight remained in the model (F = 5.2; p < 0.01), with an increase of 1.49 (CI 95%: 0.46-2.53) pg/ml with each point of resistin, an increase of 1.20 (CI 95%: 0.38-2.10) pg/ml with each 1 ng/dl of IL-6 and an increase of 0.27 (CI 95%: 0.04-0.51) pg/ml with each kg of weight. In the fifth multivariate analysis adjusted by age, sex and fat mass with a dependent variable (leptin), BMI and TNF-alpha remained in the model (F = 4.1; p < 0.01), with an increase of 10.35 (CI 95%: 4.10-21.12) pg/ml with each point of BMI and a decrease of 10.16 (CI 95%: -20.37-0.76) pg/ml with each point of TNF-alpha.

Table III: Circulating adipocytokines

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male (n = 12)</th>
<th>Female (n = 53)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>3.76 ± 2.02</td>
<td>3.97 ± 2.10</td>
<td>0.76</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>5.12 ± 3.11</td>
<td>6.81 ± 5.31</td>
<td>0.31</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>13.33 ± 9.02</td>
<td>47 ± 10.84</td>
<td>0.05</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>3.69 ± 1.78</td>
<td>4.28 ± 2.37</td>
<td>0.65</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>73.73 ± 39.71</td>
<td>150.37 ± 75.1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Discussion

The major finding of this study was that adipocytokines are related with different cardiovascular risk factors and anthropometric variables. However, these associations have a lot of different implications with each molecule and each parameter as shown in univariate and multivariate analysis.

In the literature, the most important variable that determines circulating leptin concentration is BMI, as detected in our study. In our patients, leptin concentrations are higher in females than in males, as detected by

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other authors. The relationship of leptin with TNF-alpha detected in our patients might be explain by molecular pathways. Leptin expression is regulated by the actions of TNF alpha and interleukin-1, and in conditions of inflammation. The association of leptin and resistin levels detected in our univariate analysis has not yet been described. Only, Pagano et al. have been observed a weak association between resistin and adiponectin, but not leptin and resistin levels.

Adiponectin is exclusively expressed by mature adipocytes, with increasing expression and secretion during the process of adipocyte differentiation. Males have significantly lower adiponectin levels than females, this sexual dimorphism develops during pubertal development. Our data were congruent with these previous results. Moreover, adiponectin levels are decreased in severe obesity and conditions of insulin resistance, as shown in our male group of morbid obesity patients. The adjusted relation with age and adiponectin levels detected in multivariate analysis has not a clear physiological explanation and it might be an epiphenomena.

Adiponectin decreases lipid synthesis and glucose production in the liver and causes decreases in glucose and free fatty acid concentrations in the blood. Mantzoros CS et al. have described a positive significant correlation of adiponectin levels with HDL-cholesterol and a negative with HOMA. Relationship with HOMA has been detected in our univariate analysis but in multivariate analysis the association disappeared.

Initial studies have demonstrated that obesity in mice is associated with increased circulating resistin levels. Given the incomplete homology between human and mouse resistin and the absence in humans of one of three murine resistin isoforms, resistin in humans may have a different physiologic role than that in mice without a direct relationship with insulin resistance as shown our data. Considering the expression of resistin by mononuclear cells and that obesity is a state of low-grade inflammation with activated inflammatory cascades, resistin may indeed present a molecular link between metabolic signals, inflammation, obesity and atherosclerosis.

Interleukin 6 and TNF alpha have been implicated in the regulation of energy balance and are considered potent proinflammatory mediators. Adipose tissue has been estimated that it contributes about 30% of circulating IL-6, with visceral adipose tissue producing higher levels of IL6 compared with subcutaneous tissue and is related with cardiovascular risk factors. Levels of IL-6 are responsible for the increase in acute-phase proteins, such as C-reactive protein as our data shown. TNF-alpha and IL-6 can directly lead to insulin resistance by inducing serine phosphorylation of the insulin receptor, which inhibits insulin signaling. This action might explain the relation of HOMA and IL-6 in our model.

In our population, no correlations between dietary intake and adipocytokines levels were detected. Yannakoula et al. have been described a positively association of leptin levels with energy intake from carbohydrates and negatively with dietary fat. Perhaps the difference of these results is due to different populations, morbid obese patients in our study and healthy subjects in the other. In previous studies, no significantly correlations were observed between serum adiponectin or resistin concentrations and dietary intakes, too. Perhaps, new intervention (diets, drugs or bariatric surgery) designs could elucidate these unclear relationships of adipocytokines and dietary intakes.

In conclusion, circulating adipocytokine concentrations are associated with different cardiovascular risk factors and anthropometric variables in morbid obese patients. Further studies are needed to analyze this unclear topic area with clinical and therapeutic implications.

Reference


