

Original

Resistin levels and inflammatory markers in patients with morbid obesity

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Abstract

Background: The aim of the present study was to explore the relationship of resistin levels with inflammatory markers and anthropometric parameters in morbid obese patients.

Subjects: A population of 46 morbid obese was analyzed. A complete nutritional and biochemical evaluation was performed. Patients were divided in two groups by median resistin value (3.49 ng/ml), group I (low values, average value 2.60 ± 0.5) and group II (high values, average value 5.71 ± 2.25).

Results: Patients in the group II had higher weight, BMI, fat mass, waist circumference, LDL-cholesterol, triglycerides, fibrinogen and C reactive protein than patients in group I. In the multivariate analysis with age- and sex-adjusted basal resistin concentration as a dependent variable, only fibrinogen and LDL cholesterol remained as an independent predictor in the model ($F = 8.5$; $p < 0.05$). Resistin concentration increase 0.01 ng/ml (CI95%: 0.003-0.017) for each mg/dl of fibrinogen increased. Resistin concentration increase 0.03 ng/ml (CI95%: 0.003-0.049) for each mg/dl of LDL-cholesterol increased.

Conclusion: Circulating resistin concentrations are associated with different inflammatory markers, triglycerides, LDL cholesterol and anthropometric variables in morbid obese patients. Further studies are needed to explore these interesting relationships.

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Key words: *Inflammatory markers. Morbid obesity. Resistin.*

NIVELES DE RESISTINA Y MARCADORES INFLAMATORIOS EN PACIENTES CON OBESIDAD MÓRBIDA

Resumen

Introducción: El objetivo del presente estudio es evaluar la relación entre los niveles de resistina con los marcadores inflamatorios y parámetros antropométricos en pacientes obesos morbosos.

Sujetos y métodos: Una muestra de 46 obesos morbosos fue analizada. Se realizó una valoración nutricional y bioquímica completa. Los pacientes fueron divididos en dos grupos en función de la mediana de resistina (3,49 ng/ml), grupo I (valores bajos, media del valor $2,60 \pm 0,5$ ng/ml) y grupo II (valores altos, media del valor $5,71 \pm 2,25$ ng/ml).

Resultados: Los pacientes en el grupo II presentaron un mayor peso, IMC, masa grasa, circunferencia de la cintura, LDL-colesterol, triglicéridos, fibrinógeno y proteína C reactiva que los pacientes del grupo I. En el análisis multivariante, ajustado por edad y sexo, las concentraciones basales de resistina se relacionaron con los niveles de fibrinógeno y LDL colesterol ($F = 8,5$; $p < 0,05$). Las concentraciones de resistina aumentaron 0,01 ng/ml (IC95%: 0,003-0,017) por cada mg/dl de incremento en los niveles de fibrinógeno. La resistina aumentó 0,03 ng/ml (IC95%: 0,003-0,049) por cada mg/dl que aumentaron los niveles de LDL-colesterol.

Conclusion: Los niveles circulantes de resistina se relacionan con marcadores inflamatorios, triglicéridos, LDL colesterol y datos antropométricos en pacientes con obesidad morbosos. No obstante son necesarios más estudios para evaluar estas interesantes relaciones.

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Palabras clave: *Marcadores inflamatorios. Obesidad mórbida. Resistina.*

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Introduction

Obesity is associated with cardiovascular risk factors, including altered levels of inflammatory markers and adipocytokines.¹ Obesity is characterized by a low grade systemic inflammation. Epidemiological evidence of the rising tide of obesity and associated pathologies has led, in the last years, 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.^{2,3}

Adipocytokines are proteins produced mainly by adipose tissue.⁴ Resistin is one of this adipocytokine, it was originally identified as a circulating mouse adipocyte gene product that is regulated by antidiabetic drugs.⁵ In rodents, resistin is derived exclusively from adipocytes, circulates at increased levels in obese animals and causes dysregulated hepatic glucose production, leading insulin resistance⁶⁻⁸ and appears to be a major determinant of hepatic insulin resistance induced by high-fat diet.⁹ In humans, data on the role of this adipocytokine in insulin sensitivity and obesity are controversial. A synthetic gene exists in humans, but is expressed at higher levels in monocytes and macrophages than in adipocytes.¹⁰ Some authors indicated that increased serum resistin levels are associated with increased obesity, visceral fat¹¹ and type 2 diabetes,¹²⁻¹³ while other groups failed to observe such correlations.¹⁴

Accordingly, the aim of the present study was to explore the relationship of resistin levels with cardiovascular risk factors and inflammatory markers in morbid obese patients.

Subjects and methods

Subjects

A population of 46 morbid obese (BMI > 40) patients was analyzed in a prospective way and enrolled in a consecutive population way. These patients were studied in a Nutrition Clinic Unit after signed informed consent.

Procedure

All patients with a 2 weeks weight-stabilization period before recruitment were enrolled. Weight, blood pressure, basal glucose, insulin resistance, c-reactive protein (CRP), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood and resistin levels were measured in fasting condition. 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 > 140/90 mmHg, fasting plasma glucose > 126 mg/dl, as well as the use of sulphonilurea, thiazolidinediones, insulin, glucocorticoids, antineoplastic

agents, angiotensin receptor blocker, angiotensin converting enzyme inhibitors, psychoactive medications.

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 supernatant after precipitation of other lipoproteins with dextran sulphate-magnesium. LDL cholesterol was calculated using Friedewald formula. Lipoprotein (a) was determined by immunonephelometry with the aid of a Beckman array analyzer (Beckman Instruments, Calif., USA). 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.¹⁵ CRP and fibrinogen were measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany). 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.

Anthropometric measurements and indirect calorimetry

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.¹⁶ 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.

Indirect calorimetry (MedGem; Health Tech, Golden, USA) was performed in a standard way (fasting conditions and 8 hours of previous resting). Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated.¹⁷

Statistical analysis

The results were expressed as average \pm standard deviation. The distribution of variables was analyzed

Table I
Clinical and epidemiological characteristics of study population

Characteristics	n = 46
Age (years)	48.1 ± 16.1
BMI (kg/m ²)	44.4 ± 4.4
Systolic BP (mmHg)	139.8 ± 18.2
Diastolic BP (mmHg)	83.6 ± 16.4
Glucose (mg/dl)	104.8 ± 14.5
Total cholesterol (mg/dl)	206.5 ± 36.3
LDL-cholesterol (mg/dl)	124.3 ± 31.6
HDL-cholesterol (mg/dl)	53.3 ± 11.3
Triglycerides (mg/dl)	130.6 ± 60.7
Lipoprotein(a) (mg/dl)	41.3 ± 32
Insulin (mUI/L)	22.7 ± 17.9
HOMA	5.9 ± 5.1
CRP (mg/dl)	9.7 ± 10.2
Fibrinogen (mg/dl)	434 ± 83

BMI: body mass index. BP: Blood pressure. CRP: C reactive protein.

with Kolmogorov-Smirnov test. Population was divided in two groups by resistin median. Quantitative variables with normal distribution were analyzed with a two-tailed paired Student's-t test. Non-parametric variables were analyzed with the Friedman test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A multiple regression model (step by step) was used to study the dependent variable (resistin). A p-value under 0.05 was considered statistically significant.

Results

Univariate analysis

Forty six patients gave informed consent and were enrolled in the study. The mean age was 48.1 ± 16.1 years, the mean BMI was 44.4 ± 4.3. Table I shows baseline characteristics of patients.

All subjects were weight stable during the 2 weeks period preceding the study (body weight change, 0.5 ± 0.3 kg). Anthropometric measurements showed an average waist circumference (125.5 ± 12.1 cm), waist-to hip ratio (0.94 ± 0.08), and average weight (114.8 ± 18.4 kg). Tetrapolar body electrical bioimpedance showed the next data; fat free mass (56.2 ± 16.8 kg) and fat mass (56.4 ± 16.8 kg). Indirect calorimetry showed a resting metabolic rate (RMR) (2,323.3 ± 668.4 kcal/day).

Serial assessment of nutritional intake with 3 days written food records showed a caloric intake of 1,859 ± 647 kcal/day, a carbohydrate intake of 194.1 ± 72.9 g/day, a fat intake of 79.3 ± 37.7 g/day and a protein intake of 89.6 ± 25.9 g/day.

Table II
Clinical and epidemiological characteristics of study population by median resistin value

Parameters	Group I n = 23	Group II n = 23	p
Sex (male/female)	4/19	5/18	ns
Age (years)	49.3 ± 15.8	46.9 ± 15.8	ns
Systolic BP (mmHg)	139.1 ± 13.6	136.3 ± 21.5	ns
Diastolic BP (mmHg)	83.5 ± 9.3	83.9 ± 13.3	ns
Glucose (mg/dl)	108.0 ± 17.5	102.4 ± 17.6	ns
Total cholesterol (mg/dl)	198.1 ± 43.3	206.1 ± 30.3	ns
LDL-cholesterol (mg/dl)	115.4 ± 36.5	131.2 ± 38.5	<0.05
HDL-cholesterol (mg/dl)	54.2 ± 12.9	50.1 ± 10.6	ns
Triglycerides (mg/dl)	122.6 ± 25.8	146.2 ± 13	<0.05
Lipoprotein(a) (mg/dl)	41.8 ± 26.8	41.4 ± 36.3	ns
Insulin (mUI/L)	24.8 ± 15.5	24.5 ± 20.6	ns
HOMA	6.5 ± 3.9	6.6 ± 5.8	ns
CRP (mg/dl)	6.8 ± 6.1	11.1 ± 7.1	<0.05
Fibrinogen (mg/dl)	398.3 ± 68	468.9 ± 87	<0.05

Group I (patients with the low values of median resistin) and group II (patients with the high values of median resistin). BMI: body mass index. CRP: c reactive protein. BP: Blood pressure. ns: no significant.

Patients were divided in two groups by median resistin value (3.49 ng/ml), group I (patients with the low values, average value 2.60 ± 0.5) and group II (patients with the high values, average value 5.71 ± 2.25). Table II shows the statistical differences between both groups in epidemiological and biochemical parameters. Patients in the group II had higher LDL-cholesterol, triglycerides, fibrinogen and C reactive protein than patients in group I.

Table III shows dietary intake and anthropometric parameters. Patients in the group II had higher weight, BMI, fat mass and waist circumference than patients in

Table III
Dietary intake and anthropometric characteristics by median resistin value

Parameters	Group I n = 23	Group II n = 23	p
Energy (kcal/day)	1,741 ± 346	1,899 ± 614	ns
CH (g/day)	188.2 ± 94	199.1 ± 97	ns
Fat (g/day)	77.9 ± 37	80.1 ± 46	ns
Protein (g/day)	89.3 ± 30	89.6 ± 28	ns
Weight (kg)	112.2 ± 16.1	122.4 ± 15.4	<0.05
BMI (kg/m ²)	43.3 ± 2.7	45.9 ± 5.4	<0.05
Fat free mass (kg)	55.1 ± 14.3	56.1 ± 16	ns
Fat mass (kg)	56.5 ± 11.2	58.2 ± 15.5	<0.05
Waist circumference	123.7 ± 8.6	128.1 ± 13.4	<0.05
Waist to hip ratio	0.93 ± 0.08	0.95 ± 0.09	ns
RMR (kcal/day)	2,495 ± 814	2,380 ± 787	ns

Group I (patients with the low values of median resistin) and group II (patients with the high values of median resistin). BMI: body mass index. CH: carbohydrate. RMR: resting metabolic rate. ns: no significant.

group I. No statistical differences were detected in dietary intake between both groups.

Correlation analysis showed a significant correlation among resistin levels and the independent variables; weight ($r = 0.21$; $p < 0.05$), BMI ($r = 0.16$; $p < 0.05$), LDL-cholesterol ($r = 0.33$; $p < 0.05$), triglycerides ($r = 0.18$; $p < 0.05$), fibrinogen ($r = 0.45$; $p < 0.05$) and fat mass ($r = 0.16$; $p < 0.05$).

In the multivariate analysis with age- and sex-adjusted *basal* resistin concentration as a dependent variable, only fibrinogen and LDL cholesterol remained as an independent predictor in the model ($F = 8.5$; $p < 0.05$). Resistin concentration increase 0.01 ng/ml (CI 95%: 0.003-0.017) for each mg/dl of fibrinogen increased. Resistin concentration increase 0.03 ng/ml (CI 95%: 0.003-0.049) for each mg/dl of LDL-cholesterol increased.

Discussion

The main finding of this study is that resistin levels in morbid obese patients are related with different anthropometric parameters, lipid profile and inflammatory markers.

Initial studies have demonstrated that obesity in mice, insulin resistance is associated with increased circulating resistin levels.⁷ 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. The role of resistin in the metabolic parameters is controversial, too. Some articles, reported that in humans resistin levels correlate with insulin resistance and obesity,^{5,7,18} while other investigations failed to observe any correlation of metabolic markers with resistin levels.¹⁹⁻²⁰

In a recent study,²⁰ resistin levels were correlated with fat mass, HDL cholesterol, triglycerides, c reactive protein and blood pressure, without correlation with insulin resistance, as our data shows. One explanation for the lack of correlation with insulin resistance is that many hormones affect insulin resistance, and resistin may not be a major determinant of insulin resistance. In our study, we show that resistin levels are increased by obesity and correlate with markers of inflammation. Thus, systematic inflammation leads to increased resistin production and circulating levels in human. The increased level of resistin in humans with obesity is likely an indirect result of elevated levels of inflammatory cytokines characteristics of states of increased adiposity. In univariate analysis, we observed a relation among resistin levels and fibrinogen and C reactive protein. Kunnari et al.²³ showed a positive correlation with C-reactive protein, too. These data suggest that in humans resistin could be related to the cardiovascular inflammatory state. Accordingly, the association between metabolic syndrome and resistin levels might be explain by this inflammatory

state produce by C reactive protein secondary to resistin by a direct effect without insulin resistance.²⁴ There are indications that resistin is involved in the pathogenesis of other inflammatory states such as rheumatoid arthritis. Resistin has been found in the plasma and synovial fluid of rheumatoid arthritis patients.²⁵ Qi et al.²⁶ have shown an association of resistin with inflammatory markers and fibrinolytic markers such as fibrinogen, C reactive protein and plasminogen activator inhibitor.

Other data that support the relation between inflammatory markers and resistin is the reducing effects of thiazolidinedione (TZD) class of insulin sensitizers on resistin levels and the notion that these agents could decrease CRP values is reported.²⁷ The lack of association between resistin and insulin resistance, with the presence of a relationship between resistin and inflammatory markers may be explained by a direct effect of resistin. For example, in vascular endothelial has been described this direct effect, it induces the release of endothelin 1 and other molecules that change vascular tone.²⁸

The relation of resistin with LDL cholesterol and triglycerides levels could be explained by a direct modulation of resistin expression within human white adipose tissue by cholesterol, as it was described by Jove et al.²⁹

In conclusion, circulating resistin concentrations are associated with different inflammatory markers, triglycerides, LDL cholesterol and anthropometric variables in morbid obese patients. Further studies are needed to analyze this unclear topic area with clinical and therapeutically implications, in a lot of type of patients.

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