Adipokines and genetic factors in overweight or obese but metabolically healthy Polish women

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Abstract

OBJECTIVE: Obesity may be accompanied by enhanced metabolic disturbances but not all obese patients suffer from metabolic syndrome. Since metabolic homeostasis is under control of genetic factors underlying expression of adipokines, we aimed to compare the serum concentrations of adiponectin and resistin, and polymorphism in their genes, in overweight or obese Polish women.

MATERIAL AND METHODS: The study included 265 women with BMI above 25 kg/m^2 (140 metabolically healthy and 125 with metabolic syndrome) and 104 non-obese women as a control group. Anthropometric parameters (BMI, BIA, WHR), blood pressure, lipid, glucose and HOMA-IR profiles as well as serum concentrations of adiponectin, HMW adiponectin and resistin were evaluated. Gene polymorphisms of adiponectin gene (276G/T; 11377C/G; 11391G/A) and resistin gene (420C/G; 62G/A; 537A/C) were analyzed using TaqMan SNP genotyping assays.

RESULTS: Higher serum concentrations of total adiponectin and lower levels of resistin were found in metabolically healthy patients when compared to those diagnosed with metabolic syndrome.

No differences of serum HMW and resistin concentrations were observed between overweight or obese but metabolically healthy subjects and normal weight controls.

No associations of investigated polymorphisms and the presence of metabolic syndrome were noticed in overweight/obese women with metabolic syndrome.

CONCLUSIONS: The assessment of total adiponectin in sera seems to be promising target in distinguishing subjects with obesity who undergo a diagnostic procedure for metabolic syndrome. Moreover, the evaluation of adipokine array may help to select patients with higher risk of metabolic disturbances that are associated with severe diseases.

ORIGINAL ARTICLE

INTRODUCTION

Obesity is caused by the dysfunction of energy homeostasis and disturbed appetite control that leads to increased fat mass, and in consequence, the development of multi-organ disorders. A long-term follow-up study of obese patients resulted in the definition of the metabolic syndrome in the 1980s. This syndrome is associated with visceral obesity, dyslipidemia, hypertension and malfunction of glucose metabolism (Ford & Mokdad 2008; Misra & Khurana 2008).

Dysfunction of appetite regulatory processes results in overweight and obesity. The main regulatory organ for food intake is the hypothalamus, where hunger and satiety centers are found. Neuropeptides involved in the regulation of appetite can be divided into two opposing groups: orexigenic and anorexigenic (Arora & Anubhuti 2006).

Long-term observations have confirmed that the control of appetite and energy homeostasis is influenced by a hormonal system. One of the most important hormones is insulin, which affects metabolism and the regulation of food intake. Insulin acts to increase glucose oxidation, fatty acid synthesis and amino acid uptake. On the other hand, it decreases lipolysis, lipid oxidation and gluconeogenesis. A typical feature of a large proportion of obese people is insulin resistance and hyperinsulinism. A reduction in body mass as a result of diet and/or pharmacotherapy or bariatric surgery has been found to lead to decreased insulin resistance and changes in adipokine secretion (Oda 2008; García de la Torre *et al.* 2008; Linscheid *et al.* 2008).

Since 1994, when leptin and its receptors were discovered by Friedman and co-workers, adipose tissue has no longer been considered simply as an energy reservoir. Extensive research on this tissue has revealed the existence of many substances secreted by adipocytes that have been named adipokines (or adipose-derived hormones). Moreover, the metabolic activity of the adipose tissue itself has also been confirmed. Adiponectin, its high molecular weight fraction (HMW adiponectin), resistin, visfatin and leptin are among the most important adipokines (Kershaw & Flier 2004; Vázquez-Vela *et al.* 2008).

Adiponectin is a 33-kDa polypeptide, synthesized in the adipose tissues. A rise in BMI is correlated with a decrease in the serum adiponectin concentration. Furthermore, a rise in adiponectin levels has been found to be related to a reduction in insulin resistance. Adiponectin possesses not only antidiabetic activity, but also has anti-inflammatory and anti-atherogenic properties. It was recently discovered that adiponectin may exist in different serum forms: dimer, trimer, hexamer or multimer. High molecular weight (HMW) adiponectin appears to be the most biologically active form. Data from studies on the role of HMW adiponectin suggest that elevation of the serum concentration of this form or even an increase in the HMW to total adiponectin ratio correlates positively with enhanced insulin sensitivity (Kershaw & Flier 2004; Rabe *et al.* 2008; Tilg & Moschen 2008).

Another adipokine, resistin is secreted mainly by adipocytes. It has been speculated that enhanced insulin resistance, which might be due to resistin activity, is caused by modulation of glucose metabolism (e.g. stimulation of gluconeogenesis and glycogenolysis). Resistin may also influence the deterioration of glucose utilization in skeletal muscles (Kershaw & Flier 2004; Rabe *et al.* 2008; Hivert *et al.* 2008).

It is known that obesity is correlated with many environmental and genetic factors. Studies on obese families have revealed that genetic factors impact on BMI in 30–40% of cases, whereas an environmental influence is implicated in approximately 60–70% of cases (Blakemore & Froguel 2008; Farooqui & O'Rahilly 2006). Undoubtedly, genetic factors predispose individuals to obesity, but this condition should not be considered as solely a genetic disorder.

Genetic disturbances resulting in inappropriate synthesis or malfunction of adipokines may also contribute to obesity. Polymorphisms of the adiponectin gene that are considered to be important include single nucleotide polymorphisms (SNPs) in the promotor region, SNP 45 T/G in the signal sequence and polymorphisms of introns. Such polymorphisms may increase the risk of type 2 diabetes, insulin resistance and hypertension. Thus, they may lead to the development of metabolic syndrome (Yang & Chuang 2006; Zacharova *et al.* 2005).

In addition, the most frequently identified polymorphism of resistin, 420C/G, is associated with obesity, insulin resistance and type 2 diabetes in different populations (Xu *et al.* 2007; Osawa *et al.* 2007). However, the results of studies conducted to identify the exact role of resistin gene polymorphism in the development of metabolic syndrome are still equivocal and further investigation is needed.

The aim of this study was to determine the factors influencing the incidence of metabolic syndrome in overweight or obese individuals.

MATERIAL AND METHODS

This study examined 369 women who were assigned to one of three groups:

- 125 overweight/obese women (BMI≥25 kg/m^2) with metabolic syndrome
- 140 overweight/obese women (BMI≥25 kg/m^2) without metabolic syndrome
- 104 young, healthy, lean women (BMI 18.5–24.99 kg/m^2) as a control group.

All subjects were Caucasians and were recruited from the outpatient clinic.

Metabolic syndrome was diagnosed according to the criteria of the International Diabetes Federation (2005). Hypertension was defined in accordance with the ESH and ESC criteria. A diagnosis of type 2 dia-

betes was established when fasting plasma glucose was ≥126 mg/dl or the plasma glucose concentration was ≥200 mg/dl two hours after a 75 g oral glucose load (oral glucose tolerance test, OGTT), or when the plasma glucose level anytime was ≥200 mg/dl. Diabetes classification was assessed in accordance with the criteria of the World Health Organization Experts Committee on Diabetes (1998).

Reasons for exclusion from the study included endocrine disease, chronic pulmonary dysfunction, cardiovascular disease, chronic kidney and liver diseases, and neoplasm history.

None of the examined subjects had signs of acute infectious disease at the time of the investigation. None of them was taking anti-inflammatory drugs. A history of excessive alcohol consumption and/or smoking eliminated individuals from the study.

The study protocol was accepted by the Bioethical Committee of the Medical Centre of Postgraduate Education, Warsaw.

Informed consent was obtained from all subjects.

Medical examination

Clinical data including blood pressure, height, weight and BMI were collected. Waist measurement and waist-hip ratio (WHR) were used as indicators of central obesity. Body fat content was measured by electric bioimpedance (BIA).

Peptide and hormone measurements, and assessment of lipid and glucose profiles

Blood samples were taken from all subjects in the morning after overnight (12 h) fasting. Immediately after collection, the samples were centrifuged at 4 °C, and the serum was frozen at –70 °C for later analyses:

- Adiponectin concentrations were measured by RIA (Linco Research).
- Resistin levels were measured by ELISA (Bio Vendor Laboratory Medicine).
- HMW adiponectin levels were established by EIA (ALPCO).
- Insulin concentration was measured by IRMA (BioSource).
- Serum lipid and glucose profiles were characterized using routine laboratory methods.
- Insulin resistance was estimated using the homeostasis model assessment method (HOMA-IR).
- The intra- and inter-assay coefficients were <10% for all investigated parameters.

Genotyping of selected polymorphisms of the adiponectin and resistin genes

Genomic DNA was extracted from EDTA-treated whole blood using a NucleoSpin Blood Quick Pure kit (Macharey-Nagel, Germany) in accordance with the kit protocol.

Single nucleotide polymorphisms (SNPs) of the resistin gene (420C/G, 62G/A, 537A/C) and the adiponectin gene (45T/G, 276G/T, 11377C/G, 11391G/A) were investigated using TaqMan SNP Genotyping Assays. Real Time PCR (RT-PCR) was performed with an ABI PRISM Sequence Detection System (SDS) and allelic discrimination was carried out using ABI PRISM 7000SDS software.

Statistical analyses

Statistical analyses were performed using STATISTICA version 7.1PL software. Statistical significance was accepted at $p \leq 0.05$.

The normality of distribution among the groups was investigated using the Shapiro-Wilk test and the Kolmogorow-Smirnov test with the Lilliefors correction.

The evaluation of differences between groups was performed using the Kruskal-Wallis rank test, followed by the Mann-Whitney U-test. The Spearman test was applied to calculate the correlation coefficients between adipokine levels and data from anthropometric examination and biochemical parameters.

The frequency distributions of the adiponectin and resistin polymorphism genotypes were compared using the χ^2 test. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using the online calculator available at http://www.hutchon.net/ConfidOR.htm. Evaluation was carried out for dominant model (heterozygote + recessive homozygote) *vs.* dominant homozygote.

The Hardy-Weinberg equilibrium was also estimated.

Logistic regression was used to establish associations between the analyzed adiponectin and resistin gene polymorphisms with overweight/obesity and metabolic syndrome.

In addition, OR were simultaneously evaluated.

The logistic regression was performed between the following groups: overweight/obese women with metabolic syndrome *vs.* overweight/obese women without metabolic syndrome.

RESULTS

The results of the clinical examination and biochemical parameters are shown in Table 1.

A comparison of the two groups of overweight/obese patients revealed that in women with metabolic syndrome, blood pressure (DBP and SBP), BMI, BIA, waist and hip circumferences as well as waist/hip ratio (WHR) were significantly higher than in those without metabolic syndrome ($p<0.001$ for all evaluated parameters). As expected, all parameters obtained from patients with a BMI≥25 kg/m^2 differed significantly from those of lean control subjects ($p<0.001$ for all evaluated parameters).

Not surprisingly, the serum lipid profile was found to be disturbed in the group of women with BMI≥25 kg/m^2. The highest values of total cholesterol, LDL-

Tab. 1. Clinical data, serum lipid and carbohydrate metabolism parameters and adipokine concentrations for the entire study population.

	BMI ≥25 kg/m^2 Metabolic syndrome (+) N=125	BMI ≥25kg/m^2 Metabolic syndrome (-) N=140	Control group N=104	*p*-value
Age (yrs)	46 ± 14.54	44.21 ± 16.31	28 ± 9.26	<0.001[b,c]
Systolic BP (mm Hg)	152 ± 18.84	130 ± 13.16	120 ± 19.16	<0.001[a,b,c]
Diastolic BP (mm Hg)	94 ± 9.19	82 ± 6.75	80 ± 6.21	<0.001[a,b,c]
BMI (kg/m^2)	35.17 ± 6.31	31.84 ± 5.45	21.66 ± 1.93	<0.001[a,b,c]
BIA (%)	42 ± 5.4	39 ± 5.53	27 ± 5.36	<0.001[a,b,c]
Waist (cm)	102 ± 12.67	93 ± 11.39	73 ± 6.99	<0.001[a,b,c]
Hip (cm)	118 ± 12.6	112 ± 12.22	96 ± 6.73	<0.001[a,b,c]
Waist-Hip Ratio	0.86 ± 0.06	0.83 ± 0.05	0.76 ± 0.06	<0.001[a,b,c]
Glucose (mmol/l)	6.38 ± 1.82	4.91 ± 1.31	4.2 ± 1.76	<0.001[a,b,c]
Insulin (μIU/ml)	31.18 ± 25.43	20.34 ± 24.15	11.4 ± 7.67	<0.001[a,b,c]
HOMA-IR	9.38 ± 9.74	4.55 ± 5.32	1.52 ± 1.76	<0.001[a,b,c]
Total cholesterol (mg/ml)	229 ± 43.05	202 ± 28.80	180 ± 29.79	<0.001[a,b,c]
Triglycerides (mg/ml)	191 ± 87.92	95 ± 34.20	80 ± 11.56	<0.001[a,c] <0.05[b]
HDL cholesterol (mg/ml)	52 ± 13.91	64 ± 12.94	75 ± 26.17	<0.001[a,b,c]
LDL cholesterol (mg/ml)	140 ± 39.59	119 ± 30.0	75 ± 25.17	<0.001[a,b,c]
Resistin (ng/ml)	5.64 ± 4.49	4.27 ± 1.68	3.6 ± 1.7	<0.001[a,c]
Adiponectin (μg/ml)	8.32 ± 5.01	11.15 ± 6.44	11.2 ± 5.48	<0.001[a,c]
HMW Adiponectin (μg/ml)	1.80 ± 1.05	1.94 ± 1.58	2.01 ± 1.64	NS

Data are presented as the mean ± SD
BMI ≥25 kg/m^2 Metabolic syndrome (+) - overweight or obese subjects with metabolic syndrome
BMI ≥25 kg/m^2 Metabolic syndrome (–) - overweight or obese subjects without metabolic syndrome
a - BMI ≥25 kg/m^2 Metabolic syndrome (–) *vs.* BMI ≥25 kg/m2 Metabolic syndrome (+)
b - BMI ≥25 kg/m^2 Metabolic syndrome (–) *vs.* control group
c - BMI ≥25 kg/m^2 Metabolic syndrome (+) *vs.* control group

cholesterol and triglycerides, and the lowest concentrations of HDL-cholesterol were observed in overweight or obese patients suffering from metabolic syndrome.

Insulin resistance parameters (HOMA-IR, fasting glucose and insulin concentrations) were also markedly enhanced in the overweight/obese group with diagnosed metabolic syndrome.

Serum adipokine concentrations are presented in Table 1.

Total adiponectin values remained significantly decreased in the group of women with BMI≥25 kg/m^2 and metabolic syndrome in comparison with the metabolically healthy BMI≥25 kg/m^2 group or the controls (p<0.001, p<0.001). However, no difference was found between the control group and overweight/obese subjects without metabolic syndrome.

HMW adiponectin levels did not differ between the three groups.

The lowest concentrations of resistin were found in the control group, but they did not differ significantly from those of metabolically healthy subjects with BMI≥25 kg/m^2. However, resistin levels were significantly higher in women with overweight/obesity and metabolic syndrome when compared with the other groups (p<0.001, p<0.001).

A number of important correlations were identified in overweight/obese women:

Adiponectin concentrations correlated negatively with insulin resistance markers (insulin and HOMA-IR) in both groups of women with overweight/obesity. A negative correlation between total adiponectin and markers of visceral obesity (waist circumference and WHR) was observed in women with BMI≥25 kg/m^2 and metabolic syndrome, but not in the metabolically healthy overweight/obese women.

There was also a negative correlation between HMW adiponectin and insulin, HOMA-IR (but only in the metabolic syndrome-negative group) and visceral obesity indicators (waist circumference in both groups, and WHR only in individuals with metabolic syndrome).

Resistin correlated positively with BMI, BIA, waist and hip circumferences, but only in women diagnosed with metabolic syndrome.

All other correlations are shown in detail in Tables 2A and 2B.

Statistical analysis did not detect any significant differences in the frequency of the investigated resistin and adiponectin gene polymorphisms in any of the groups studied. In the control group, the genotype distributions for all polymorphisms were in Hardy-Weinberg equilibrium. OR (95% CI) for the dominant model did not identify any significant differences between patients with BMI≥25 kg/m^2 diagnosed with metabolic syndrome and metabolically healthy overweight/obese subjects. However, OR were markedly different for the adiponectin gene SNP 276G/T and resistin gene SNP420 C/G, when overweight/obese women with metabolic syndrome were compared with lean controls.

The estimation of association of the analyzed polymorphisms in the adiponectin and resistin genes with overweight/obesity, with or without associated metabolic syndrome, did not reveal any significant differences.

Genotype frequencies and OR are presented in Tables 3, 4 and 5.

Tab. 2. Statistically significant correlations between serum adipokines and clinical/biochemical parameters in women with BMI ≥25 kg/m^2 and metabolic syndrome (**A**) and metabolically healthy obese women (**B**).

A

Adipokines		R	*p*-value
Adiponectin	BMI	–0.14	<0.05
Adiponectin	Systolic BP	–0.18	=0.06
Adiponectin	Waist	–0.19	<0.05
Adiponectin	WHR	–0.38	<0.001
Adiponectin	Insulin	–0.30	<0.001
Adiponectin	HOMA-IR	–0.27	<0.01
Adiponectin	HDL-cholesterol	+0.30	<0.01
Adiponectin	Triglycerides	–0.27	<0.01
HMW Adiponectin	Systolic BP	–0.28	<0.05
HMW Adiponectin	BMI	–0.27	<0.05
HMW Adiponectin	Waist	–0.32	<0.05
HMW Adiponectin	WHR	–0.36	<0.05
HMW Adiponectin	Insulin	–0.23	=0.07
HMW Adiponectin	HDL-cholesterol	+0.34	<0.01
Resistin	BMI	+0.33	<0.001
Resistin	BIA	+0.27	<0.01
Resistin	Waist	+0.25	<0.01
Resistin	Hip	+0.35	<0.001

B

Adipokines		R	*p*-value
Adiponectin	Insulin	–0.22	<0.05
Adiponectin	HOMA-IR	–0.21	<0.05
Adiponectin	HDL-cholesterol	+0.22	<0.05
HMW Adiponectin	BMI	–0.27	<0.05
HMW Adiponectin	Waist	–0.24	<0.05
HMW Adiponectin	Insulin	–0.45	<0.001
HMW Adiponectin	HOMA-IR	–0.35	<0.01
HMW Adiponectin	HDL-cholesterol	+0.39	<0.05
Resistin	HDL-cholesterol	–0.25	<0.05

DISCUSSION

Population studies indicate that some overweight or obese subjects do not show enhanced metabolic disturbances. This particular group is called overweight/obese but metabolically healthy. The exact criteria for distinguishing metabolically healthy subjects have not yet been precisely defined (Karelis 2008). However, these individuals are characterized by relatively high insulin sensitivity, normal lipid profiles and normotension (Karelis 2008). It has been estimated that even in a group with severe obesity (BMI≥40 kg/m^2), up to 30% of subjects are metabolically healthy. Moreover, clinical studies indicate that obese but metabolically healthy subjects have a lower risk of cardiovascular disease than those of equivalent weight with metabolic syndrome (Karelis 2008; Marini *et al.* 2007).

Both experimental and clinical investigations have revealed that abdominal adipose tissue, in particular visceral tissue, exhibits different metabolic properties

Tab. 3. OR with 95% CI for adiponectin and resistin gene polymorphisms in women with BMI ≥25 kg/m^2 suffering from metabolic syndrome *vs.* metabolically healthy obese women.

Polymorphism	Genotype	BMI ≥25 kg/m^2 Metabolic syndrome (+)	BMI ≥25 kg/m^2 Metabolic syndrome (–)	OR and 95% CI BMI ≥25 kg/m^2 Metabolic syndrome (+) *vs.* BMI ≥25 kg/m^2 Metabolic syndrome (–)	*p*-value
Adiponectin 276 G/T	GT+TT GG	N = 71 27 (38.03%) 44 (61.97%)	N = 105 53 (50.48%) 52 (49.52%)	0.60 (0.33–1.11)	NS
Adiponectin 11377 C/G	CG+GG CC	N = 77 33 (42.86%) 44 (57.14%)	N = 114 53 (46.49%) 61 (53.51%)	0.86 (0.48–1.55)	NS
Adiponectin 45 T/G	GT+GG TT	N = 57 9 (15.79%) 48 (84.21%)	N =73 9 (12.33%) 64 (87.67%)	0.75 (0.28–2.03)	NS
Adiponectin 11391 G/A	GA+AA GG	N = 72 14 (19.44%) 58 (80.56%)	N = 110 26 (23.64%) 84 (76.36%)	1.28 (0.62–2.66)	NS
Resistin 420 C/G	CG+GG CC	N = 68 27 (39.71%) 41 (60.29%)	N = 104 51 (49.04%) 53 (50.96%)	1.46 (0.78–2.71)	NS
Resistin 62 G/A	GA+AA GG	N =71 6 (8.45%) 65 (91.55%)	N = 106 12 (11.32%) 94 (88.68%)	1.38 (0.49–3.87)	NS
Resistin 537 A/C	AC+CC AA	N = 76 5 (6.58%) 71 (93.42%)	N = 114 14 (12.28%) 100 (87.72%)	1.99 (0.69 – 5.69)	NS

Tab. 4. OR with 95% CI for adiponectin and resistin gene polymorphisms in women with BMI ≥25 kg/m^2 suffering from metabolic syndrome *vs.* lean control group.

Polymorphism	Genotype	BMI ≥25 kg/m^2 Metabolic syndrome (+)	Control group	OR and 95% CI BMI ≥25 kg/m^2 Metabolic syndrome (+) *vs.* control group	*p*-value
Adiponectin 276 G/T	GT+TT GG	N = 71 27 (38.03%) 44 (61.97%)	N = 52 31(59.62%) 21 (40.38%)	0.41 (0.20–0.86)	<0.05
Adiponectin 11377 C/G	CG+GG CC	N = 77 33 (42.86%) 44 (57.14%)	N =55 31 (56.36%) 24 (43.64%)	0.58 (0.29–1.16)	NS
Adiponectin 45 T/G	GT+GG TT	N = 57 9 (15.79%) 48 (84.21%)	N = 39 4 (10.26%) 35 (89.74%)	0.69 (0.17–2.13)	NS
Adiponectin 11391 G/A	GA+AA GG	N = 72 14 (19.44%) 58 (80.56%)	N = 53 9 (16.98%) 44 (83.02%)	0.87 (0.33–2.16)	NS
Resistin 420 C/G	CG+GG CC	N = 68 27 (39.71%) 41 (60.29%)	N = 51 30 (58.82%) 21 (41.18%)	2.16 (1.04–4.52)	<0.05
Resistin 62 G/A	GA+AA GG	N =71 6 (8.45%) 65 (91.55%)	N = 52 6 (11.54%) 46 (88.46%)	1.41 (0.42–4.68)	NS
Resistin 537 A/C	AC+CC AA	N = 76 5 (6.58%) 71 (93.42%)	N = 55 6 (10.91%) 49 (89.09%)	1.73 (0.50–5.94)	NS

Tab. 5. OR with 95% CI for adiponectin and resistin gene polymorphisms in women with BMI ≥25 kg/m^2 without metabolic syndrome *vs.* lean control group.

Polymorphism	Genotype	BMI ≥25 kg/m^2 Metabolic syndrome (–)	Control group	OR and 95% CI BMI ≥25 kg/m^2 Metabolic syndrome (–) *vs.* control group	*p*-value
Adiponectin 276 G/T	GT+TT GG	N = 105 53 (50.48%) 52 (49.52%)	N = 52 31(59.62%) 21 (40.38%)	0.69 (0.35–1.35)	NS
Adiponectin 11377 C/G	CG+GG CC	N = 114 53 (46.49%) 61 (53.51%)	N =55 31 (56.36%) 24 (43.64%)	0.67 (0.35–1.28)	NS
Adiponectin 45 T/G	GT+GG TT	N =73 9 (12.33%) 64 (87.67%)	N = 39 4 (10.26%) 35 (89.74%)	0.81 (0.23–2.82)	NS
Adiponectin 11391 G/A	GA+AA GG	N = 110 26 (23.64%) 84 (76.36%)	N = 53 9 (16.98%) 44 (83.02%)	0.66 (0.29–1.53)	NS
Resistin 420 C/G	CG+GG CC	N = 104 51 (49.04%) 53 (50.96%)	N = 51 30 (58.82%) 21 (41.18%)	1.48 (0.75–2.91)	NS
Resistin 62 G/A	GA+AA GG	N = 106 12 (11.32%) 94 (88.68%)	N = 52 6 (11.54%) 46 (88.46%)	1.02 (0.36–2.89)	NS
Resistin 537 A/C	AC+CC AA	N = 114 14 (12.28%) 100 (87.72%)	N = 55 6 (10.91%) 49 (89.09%)	0.87 (0.32–2.36)	NS

compared with such tissue in gynoidal lower body type obesity (Hajer *et al.* 2008; Aguilera *et al.* 2008). Visceral adipose tissue has higher lipolytic activity and shows enhanced release of free fatty acids in the case of hyperinsulinism and insulin resistance. In addition, abdominal adipocytes are resistant to the antilipolytic activity of insulin (Rasouli & Kern 2008; Lee *et al.* 2009).

A new hypothesis concerning the activities of visceral adipose tissue is currently under consideration. This proposes that this tissue may represent an ectopic deposit for excess fat, similar to the storage of triglycerides in myocytes or hepatocytes. Dysfunction of this process may lead to decreased accumulation of lipids by subcutaneous adipose tissue (Hajer *et al.* 2008; Aguilera *et al.* 2008, Rasouli & Kern 2008).

In the present study, serum adiponectin concentrations were found to be decreased in a group of overweight/obese women with metabolic syndrome in comparison with similar subjects without metabolic disturbances. It is also worth noting that adiponectin levels in the latter group were lower than in lean controls, although the differences were not statistically significant. It has previously been reported that the main cause of reduced adiponectin levels in subjects suffering from obesity associated with metabolic syndrome is the coexistence of visceral obesity and insulin resistance. This observation was made following comparisons with obese metabolically healthy subjects and those whose weight is within the normal range (Tilg & Moschen 2008; Hivert *et al.* 2008; Lee *et al.* 2009).

Our results also revealed a negative correlation between adiponectin – both total and its high molecular weight form – and parameters of insulin resistance. This correlation was found in the two groups with BMI above the normal range. However, lower values of insulin resistance parameters and decreased signs of abdominal obesity were observed when metabolically healthy overweight and obese women were compared with those diagnosed with metabolic syndrome.

Adiponectin, including its HMW form, has been shown to possess anti-inflammatory properties, but its association with regulatory mechanisms of the inflammation process remains unclear (Kadowaki *et al.* 2008; Kim *et al.* 2007). It has been suggested that adiponectin may stimulate cyclooxygenase 2 (COX2) activity in cardiomyocytes that leads to an increase in prostaglandin E_2 levels and a decrease in TNFα concentration, which suppress inflammatory processes in the adipose tissue (Shibata *et al.* 2005).

We found markedly higher resistin levels in women with BMI≥25 kg/m^2 with metabolic syndrome in comparison with metabolically healthy overweight/obese subjects. Moreover, analyses of correlations between resistin and anthropometric and biochemical param-

eters showed that only markers of obesity (BMI, BIA, waist and hip circumferences) correlated with resistin, and this was the case only in subjects with metabolic syndrome. No correlations between resistin and insulin resistance markers were identified in any of the examined groups.

It has been suggested that resistin may interfere with insulin resistance, obesity and type 2 diabetes. The findings of experimental animal studies indicate that there is a connection between the rise in resistin levels and enhanced insulin resistance. However, data from investigations conducted on humans remain equivocal (Kershaw & Flier 2004; Vázquez-Vela *et al.* 2008). Previous studies have shown that the synthesis of resistin occurs mainly in visceral adipose tissue, and only small amounts are produced by subcutaneous fat [(Kershaw & Flier 2004; Vázquez-Vela *et al.* 2008; Rabe *et al.* 2008). In addition, resistin synthesis is thought to be influenced by inflammation, as it was reported that proinflammatory factors (eg. TNFα, IL-6, CRP) enhance the production of this peptide. On the other hand, it has also been reported that resistin causes an increase in IL-6 and TNFα expression in white adipose tissue (Kershaw & Flier 2004; Vázquez-Vela *et al.* 2008; Lehrke *et al.* 2004).

These data from the literature as well as our results demonstrating significant correlations between adiponectin and parameters of insulin resistance indicate that serum adiponectin – both total and HMW – may be a good marker of metabolic activity of the adipose tissue. This appears to be an even better indicator than estimation of the resistin concentration. Thus, evaluation of adiponectin may be included among the criteria used to distinguish overweight or obese, but metabolically healthy patients.

Our examination of selected adiponectin gene polymorphisms did not identify any significant differences in Odds Ratios between groups of overweight or obese women suffering from metabolic syndrome and metabolically healthy subjects.

The results of previous studies on the influence of adiponectin gene polymorphisms on adiponectin blood concentration and the impact of these polymorphisms on the risk of type 2 diabetes, insulin resistance or hypertension are equivocal (Yang & Chuang 2006). Adiponectin SNP 276G/T may be significant in enhancing the risk of disease associated with reduced adiponectin concentrations. The presence of allele G instead of allele T in the genotype was reported to cause hypoadiponectinemia, and may be linked with a higher risk of type 2 diabetes (Hara *et al.* 2002; Stumvoll *et al.* 2002). However, studies conducted in Taiwan on people over 65 years old revealed that the presence of allele G of adiponectin SNP 276G/T was correlated with lower incidences of obesity, metabolic syndrome and diabetes (Yang *et al.* 2007). On the other hand, Polish investigations on the role of adiponectin SNPs 11391G/A and 276G/T in the development of type 2 diabetes showed the opposite association (Szopa *et al.* 2009). It is noteworthy that even studies on homogenous populations have given contradictory results (Yang & Chuang 2006).

Another adiponectin gene polymorphism that could possibly be associated with an enhanced risk of type 2 diabetes and metabolic syndrome is SNP 45T/G. In addition, this polymorphism may coexist with SNP 276G/T (Zacharova *et al.* 2005; Menzaghi *et al.* 2002; Zhang *et al.* 2008). Moreover, a previous study indicated that SNP 276G/T and SNP 11377C/G may enhance the risk of severe obesity (Bouatia-Naji *et al.* 2006).

In the present study we found no significant differences in the Odds Ratios for adiponectin SNPs 45G/T, 11391G/A and 11377C/G between any of the investigated groups. However, it was found that the presence of allele T, both as the homozygote TT or heterozygote GT, in the SNP 276G/T polymorphism may be associated with a 2.5-fold lower risk of obesity with metabolic syndrome, when these patients were compared with lean controls.

We also examined the OR of 3 resistin gene polymorphisms and found significant differences in that of SNP 420C/G when women with BMI≥25 kg/m^2 suffering from metabolic syndrome were compared with lean controls. However, no further differences in OR were observed in other comparisons. Our data for this SNP also indicated that there is a co-occurrence of genotypes GG or CG and an increased risk of obesity with metabolic syndrome.

Similarly to studies on adiponectin gene polymorphisms, the data concerning the clinical application of resistin gene polymorphisms remain unclear. One of the most investigated polymorphisms of the resistin gene is RETN-420C/G (SNP 420C/G). The presence of this particular polymorphism, with the genotype GG or CG, in a Chinese study population was associated with an increase in the rate of coronary disease compared with subjects possessing the CC genotype (Tang *et al.* 2008). In contrast, this polymorphism had no apparent influence on the risk of coronary heart disease, myocardial infarction or renal failure in a study conducted in Italy (Norata *et al.* 2007). Nonetheless, a Japanese study revealed that there may be an association between resistin SNP 420C/G and reduced levels of HDL-cholesterol and an increase in CRP, two markers of an enhanced risk of cardiovascular disease (CVD) (Osawa *et al.* 2007).

A five-year follow-up study of a southern Chinese population found that the presence of allele A in SNP 62G/A and allele G in SNP 420C/G may be associated with higher serum glucose levels (Xu *et al.* 2007). Furthermore, a German study did not confirm the observation that resistin SNP 62G/A is associated with the risk of type 2 diabetes, although an association between this polymorphism and hypertension was found (Gouni-Berthold *et al.* 2005). It has been reported that resistin SNPs 537A/G and 420C/G may influence serum resistin levels, but this study failed to identify any association between these polymorphisms and the risk of type 2 diabetes (Cho *et al.* 2004).

Clinical observations of the occurrence of obesity and its relationship with resistin gene polymorphisms are ambiguous. It has been reported that Brazilian women with the CC genotype in resistin SNP 420C/G have a much higher BMI than women possessing the G allele (Mattevi *et al.* 2004). In addition, Canadian males with the GG genotype in SNP 420C/G had a relatively smaller amount of visceral adipose tissue and showed a lower insulin rise during an oral glucose tolerance test (Bouchard *et al.* 2004). However, these results were not replicated in the female group examined in the same study.

Taken together, these data suggest that both ethnic origin and gender influence the clinical features and biochemical parameters found in individuals with heterogeneous resistin gene polymorphism genotypes.

A limitation of the present study is the difference in age range between the controls and the obese/overweight subjects. It is difficult to predict how many and which of the young, lean women of the control group will become obese or overweight. Thus, prospective studies are required to resolve this problem.

To summarize, the presence of the adiponectin and resistin polymorphisms investigated in this study might cause some genetic predisposition to obesity and metabolic syndrome. However, these polymorphisms should not be used as markers to differentiate obesity that is associated with metabolic syndrome from metabolically healthy obesity.

In conclusion, the assessment of total serum adiponectin seems to be a promising way of identifying obese/overweight subjects who also have metabolic syndrome. Moreover, the evaluation of adipokines may help to select patients with a higher risk of metabolic disturbances that are associated with severe conditions including cardiovascular disease.

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