Obesity Facts
The European Journal of Obesity

Obes Facts 2013;6:393–404
DOI: 10.1159/000354574
Received: August 5, 2012
Accepted: October 25, 2012
Published online: August 16, 2013

1662–4033/13/0064–0393$38.00/0
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Original Article

Correlation of Resistin with Inflammatory and Cardiometabolic Markers in Obese Adolescents with and without Metabolic Syndrome

Emna Makni[a] Wassim Moalla[a, b] Lamia Benezzeddine-Boussaidi[a]
Gérard Lac[c] Zouhair Tabka[a] Mohammed Elloumi[a, c]

[a]Laboratory of Cardiocirculatory, Respiratory, and Hormonal Adaptations to Muscular Exercise, Faculty of Medicine Ibn El Jazzar, University of Sousse, Sousse, [b]UR EM2S, ISSEP Sfax, Tunisia, [c]Laboratory AME2P, EA 3533, BP 10448, Clermont University Blaise Pascal, Clermont-Ferrand, France

Key Words
Resistin · Metabolic syndrome · Insulin resistance · Childhood obesity · Inflammation

Abstract
Objective: The link between plasma resistin and obesity-related cardiometabolic disorders in children remains debatable. This study assessed the relationships of plasma resistin with cardiovascular risk factors, pro-inflammatory markers and insulin resistance index (HOMA-IR) in obese (Ob) adolescents and obese adolescents with metabolic syndrome (Ob-MS) compared to healthy controls (CO). ***Methods:*** 114 obese adolescents (60 Ob, age 13.6 ± 0.9 years, BMI 28.0 ± 2.2 kg/m^2, and 54 Ob-MS, age 13.8 ± 1.0 years, BMI 32.5 ± 4.8 kg/m^2) and 37 CO (age 13.7 ± 0.8 years, BMI 22.8 ± 0.8 kg/m^2) were studied. Anthropometrics, cardiac variables as well as fasting plasma concentrations of lipids, glucose, insulin, and adipocytokines (resistin, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP)) were measured. HOMA-IR was calculated, and the presence of MS was assessed. ***Results:*** Plasma resistin was significantly higher in Ob-MS than in both Ob and CO and was correlated with anthropometric, cardiovascular, pro-inflammatory markers and several components of MS as was HOMA-IR in Ob and Ob-MS. With increasing the number of MS components, plasma resistin, pro-inflammatory markers, and HOMA-IR were also increased. Multiple regression models highlighted significant correlation between resistin and both HOMA-IR ($r = 0.40$, $p < 0.05$) and systolic blood pressure ($r = 0.63$, $p < 0.01$) in Ob-MS. ***Conclusion:*** These results support the hypothesis that there is an association between circulating resistin and childhood obesity-related inflammatory and cardiometabolic events.

Mohamed Elloumi
Laboratory of Cardiocirculatory, Respiratory, and Hormonal Adaptations to Muscular Exercise
Faculty of Medicine Ibn El Jazzar, University of Sousse, 4000 Sousse (Tunisia)
elloumimed@yahoo.fr

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DOI: 10.1159/000354574

Introduction

Metabolic syndrome (MS) is described as a combined occurrence of atherogenic dyslipidemia, insulin resistance, elevated blood pressure, and central adiposity [1–3]. Obesity is a key component in the development of the MS [4, 5]. Both obesity and MS have the potential to influence the incidence and severity of cardiovascular risk factors with serious implications for worldwide health care systems, thus becoming a major public health challenge [3–7]. Increasing obesity categories in children and adolescents were associated with worsening of all components of the MS. Specifically, not only an increase in fasting glucose, fasting insulin, triglycerides and systolic blood pressure but also the prevalence of impaired glucose tolerance and a decrease of high-density lipoprotein-cholesterol (HDL-C) were observed as the degree of obesity rose [8]. Molecular approaches of the mechanism by which accumulation of body fat triggers various pathological conditions are not clearly understood. However, genes that are specially expressed in adipose tissue have been identified by large-scale random sequence analysis and have been mapped. In addition, adipose tissue is currently considered as an endocrine organ and secretes a variety of products that participates in energy homeostasis and physiological function such as insulin sensitivity, immunity and inflammation [6, 9, 10]. Secreted adipokines from adipose tissue, including adiponectin, leptin, resistin and visfatin, as well as cytokines and chemokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and the hepatic pro-inflammatory C-reactive protein (CRP) contribute to the pathophysiology of obesity-linked disorders [9–12]. Resistin is a 12.5 kDa polypeptide hormone produced by adipocytes and immune competent cells. It belongs to the resistin-like family that was originally discovered as a result of examining differential gene expression of mouse adipose tissue after thiazolidinedione treatment [13]. In human studies, the link between resistin and fatness regulation and insulin resistance remains controversial due to inconsistent findings in different studies [12, 14, 15]. Accordingly, in adult subjects, plasma resistin was reported to be related to fat mass [16] and to insulin resistance [17, 18] while other reports showed the lack of such correlations with BMI, percentage of body fat (%BF) [17], or insulin sensitivity [14]. Conversely, several youth obese studies suggested that resistin is strongly associated with central obesity but is weakly implicated in a concentration-dependent fashion in any obesity-related metabolic disorders [19, 20]. Specifically, resistin being identical to FIZ3 (= 'found in inflammatory zone 3') has been linked with inflammation [21]. It was positively correlated with pro-inflammatory factors in adults and with pathophysiological conditions such as atherosclerosis, renal diseases, and inflammation of the respiratory tract [22]. Pro-inflammatory molecules such as TNF-α, IL-6, and lipopolysaccharide regulate resistin gene expression in various cell models [23] , and reciprocal modulation has been hypothesized [24].

Given the crucial role of childhood obesity in the pathogenesis of MS and the insufficient knowledge of the underlying pathophysiological mechanisms and pathways concerning the link between plasma resistin and insulin resistance, cardiovascular disease as well as inflammation in obese children, we tested the hypothesis that plasma levels of resistin could play a significant role in energy homeostasis and development of cardiovascular disease in obese children through a central or peripheral mechanism in relation to the presence of MS components. Moreover, we examined whether or not high levels of resistin are linked to low-grade systemic inflammation in obese adolescents and those with MS when compared to their age-, sex-, and puberty-matched healthy controls. The aim of this study was therefore to investigate the relationship between plasma resistin and anthropometric variables, inflammatory and cardiovascular risk factors, HOMA-IR as well as the presence of MS in obese adolescents and if there are any differences to matched healthy controls.

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Material and Methods

Participants' Recruitment

114 obese adolescents were selected from a random sample of college students. The participants were selected by a two-level cluster sampling – i) three secondary schools among 50 were chosen randomly from the center of Tunisia; ii) the sample includes a mix of rural and urban children – and by a standard questionnaire [25]. The following exclusion criteria were respected: i) respiratory symptoms in the last 4 months before testing; ii) cardiac disease or any acute or chronic respiratory disease such as chronic cough, chronic congestion or phlegm, wheezing, or asthma; iii) chest, abdominal or nasal surgery; and iv) smoking. A total of 114 obese adolescents (60 males and 54 females) whose BMI was above the 97th percentile were studied [26]. They were classified according to the presence of MS characteristics: obese without MS (Ob) and obese with MS (Ob-MS). 37 (19 males and 18 females) age-, sex- and puberty-matched healthy controls (CO) were also included from the same randomized college students. The study was performed with the agreement of the Ministries of Education and Public Health and had received approval from local ethics committee of Farhat Hached Hospital of Sousse, Tunisia, in accordance with the ethical standards of the Helsinki Declaration of 1975. Prior to the study, the adolescents and their parents were informed about the experimental procedures and the associated risks as well as about the benefits of participation and gave their written informed consent.

Measurements

Anthropometric and Cardiovascular Data

Medical examination and anthropometric measurements for each subject were performed by a pediatrician. Body mass was measured to the nearest 0.1 kg on a digital scale (OHAUS, Florham Park, NJ, USA). Height was measured with a standing stadiometer and recorded with a precision of 0.1 cm. The BMI was calculated as body weight (kg) divided by the square of height (m). The Z-score for BMI was calculated according to the formula given by Rolland-Cachera et al. [26]. The percentage of body fat was calculated using the equation of Slaughter et al. [27] as described in the following:

If triceps and subscapular skinfolds are <35 mm:

Boys: %BF = 1.21 (sum of 2 skinfolds)^2 – 0.008 (sum of 2 skinfolds) – 1.7

Girls: %BF = 1.33 (sum of 2 skinfolds)^2 – 0.013 (sum of 2 skinfolds) – 2.5

If triceps and subscapular skinfolds are >35 mm:

Boys: %BF = 0.783 (sum of 2 skinfolds) – 1.7

Girls: %BF = 0.546 (sum of 2 skinfolds) + 9.7

Waist and hip circumferences were measured using a ribbon meter, and both waist/hip and waist/height ratios were calculated.

Pubertal stages were evaluated according to the Tanner classification [28] by the same trained pediatrician and classified into two categories: Pubertal adolescents included subjects in stage II–III, and postpubertal adolescents included subjects in stage IV–V.

Systolic and diastolic blood pressure (BP) were measured twice with the child at rest in seated position by auscultation with an aneroid sphygmomanometer (Richter, Germany, Hamburg, Germany) and appropriate-size cuff following 5-min of quiet rest. The average of these two recordings was used for further analysis.

Biochemical Parameters

Blood samples were obtained between 7:00 a.m. and 8:00 a.m. after an overnight fast (minimum 12 h). Samples were collected in EDTA-containing tubes and immediately centrifuged at 4 °C. Plasma samples were kept on dry ice during transportation from the testing sites and were stored at −80 °C until analyses.

Plasma resistin was measured by an enzyme-linked immunoassay kit obtained from Biovendor Laboratory Medicine Inc. (Brno, Czech Republic). The intra- and inter-assay coefficients of variability (CV) were 4.5 and 7.8%, respectively. Plasma levels of IL-6 and TNF-α were measured with saline using Quantikine ELISA kits from R&D Systems Inc. (Minneapolis, MN, USA; catalogue nos. HS600 or HSTA00C). The intra-assay CV were <10% for both TNF-α and IL-6. The inter-assay CV was 12.2 and 18.2% for TNF-α and IL-6, respectively. CRP concentration was measured by a high-sensitivity particle-enhanced turbidimetric assay

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using a validated commercial hsELISA kit from Kalon Biological (Kalon Biological, Guilford, UK). The intra- and inter-assay CV were 5.6 and 7.6%, respectively. Triglycerides, high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels were measured in all subjects using standardized techniques [29]. ApolipoproteinA-I (ApoA-I) and apolipoproteinB (ApoB) levels were measured with a Behring nephelometer using Behring reagents (Behring Diagnostic, Inc., Somerville, NJ, USA) and calibrated with the Northwest Lipid Research laboratories calibrator. Plasma glucose concentrations were measured using an automated device (AU2700, Olympus, Paris, France). The inter-assay CV was 1.7%. Plasma insulin was assayed by an IRMA Insulin kit (Beckman Coulter – Immunotech, Marseille, France). The intra- and inter-assay CV were 3.3–4.0 and 3.7–4.8%, respectively. Insulin resistance index was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) as: (fasting insulin in μU/l) × (fasting glucose in mmol/l) / 22.5 [30]. The presence of pediatric MS was defined according to the criteria provided by the International Diabetes Federation, and classified by the presence of two or more of the following components [31]: i) systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg; ii) triglycerides ≥ 1.7 mmol/l; iii) HDL-C < 1.03 mmol/l; and/or iv) fasting glucose ≥ 5.6 mmol/l in addition to waist circumference ≥ 90th percentile.

Statistical Analyses

Data are presented as means ± SD. The normality of distribution was checked for all parameters with the Kolmogorov-Smirnov test. Differences between subjects, who were categorized into the three groups according to the presence or absence of MS, were assessed using analysis of covariance with Scheffe' post hoc tests. Differences in natural logged size-adjusted resistin and HOMA-IR data across the three groups were determined using ANCOVA models, with adjustment for fat mass, BMI, and BMI z-score. Spearman's correlation analyses were used to analyze the relationships between anthropometric, cardiovascular risk factors as well as inflammatory variables and resistin or HOMA-IR. Multiple regression analyses were conducted to determine the predictor for resistin after adjusting for the confounding variables including age, sex, and pubertal stage. Analysis was performed using StatView software, and the significance threshold was set at $p < 0.05$.

Results

Anthropometric and Biochemical Data

Anthropometric and cardiac variables as well as pro-inflammatory and biochemical parameters of the study participants are summarized in table 1. In Ob and Ob-MS, all anthropometric data were significantly higher than in CO (all $p < 0.001$), except for height and waist/hip ratio. In addition, all anthropometric variables were significantly higher in Ob-MS than in Ob ($0.05 < p < 0.001$). Triglycerides, ApoB, ApoB/ApoA-I ratio, insulin, resistin, TNF-α, IL-6, CRP, systolic and diastolic BP, and HOMA-IR were significantly higher while ApoA-I and HDL-C were significantly lower in the Ob-MS than in Ob ($0.01 < p < 0.001$) and CO (all $p < 0.001$). These differences were also true in Ob compared to CO (all $p < 0.001$). No significant differences between groups were observed in total cholesterol and LDL-C.

We evaluated the means of plasma resistin, TNF-α, IL-6, CRP, and HOMA-IR levels within groups based on the number of MS components. All these markers were significantly increased as a function of the number of MS components from CO to Ob-MS ($0.05 < p < 0.001$; fig. 1).

Correlations between Resistin and Both Anthropometric and Biological Variables

Correlations between resistin and both anthropometric and biological variables in the three groups (CO, Ob and Ob-MS) are summarized in table 2.

In Ob-MS, plasma resistin levels showed significant correlations with BMI z-score, waist and hip circumferences, waist/height ratio, ApoA-I, ApoB/ApoA-I ratio, insulin, systolic and diastolic BP, TNF-α, IL-6, and CRP. Additionally, we discovered a significant correlation between resistin and HOMA-IR.

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Table 1. Physical characteristics and biochemical data

	CO (n = 37)	Ob (n = 60)	Ob-MS (n = 54)
Male/female	19/18	24/36	33/21
Tanner stages (II–III/IV-V)	17/20	30/30	18/36
Age, years	13.7 ± 0.8	13.6 ± 0.9	13.8 ± 1.0
Weight, kg	62.3 ± 4.5	71.4 ± 7.5[a]	88.9 ± 16.1[a, ***]
Height, m	1.65 ± 0.05	1.60 ± 0.06[a]	1.65 ± 0.06 ***
BMI, kg/m^2	22.8 ± 0.8	28.0 ± 2.2[a]	32.5 ± 4.8[a, ***]
BMI z-score	1.9 ± 0.2	3.0 ± 0.5[a]	4.1 ± 1.1[a, ***]
Fat mass, kg	18.5 ± 2.6	28.3 ± 6.2[a]	37.4 ± 7.7[a, ***]
Waist circumference, cm	66.4 ± 5.7	93.9 ± 5.7[a]	108.3 ± 10.1[a, ***]
Hip circumference, cm	73.1 ± 5.2	105.0 ± 6.3[a]	114.0 ± 9.3[a, ***]
Waist/hip ratio	0.91 ± 0.05	0.90 ± 0.05	0.95 ± 0.05[a, ***]
Waist/height ratio	0.40 ± 0.04	0.59 ± 0.04[a]	0.66 ± 0.06[a, ***]
Systolic BP, mm Hg	120.2 ± 5.2	128.4 ± 5.3[a]	136.0 ± 4.9[a, ***]
Diastolic BP, mm Hg	65.1 ± 7.4	77.0 ± 3.5[a]	82.8 ± 4.9[a, ***]
Glucose, mmol/l	2.7 ± 0.5	5.0 ± 0.8[a]	4.9 ± 0.9[a]
Insulin, µU/l	8.4 ± 2.4	20.5 ± 4.5[a]	25.4 ± 5.0[a, ***]
HOMA-IR	1.1 ± 0.4	4.6 ± 1.2[a]	5.5 ± 1.4[a, **]
Triglycerides, mmol/l	0.9 ± 0.3	1.3 ± 0.4[a]	1.7 ± 0.2[a, ***]
Total cholesterol, mmol/l	4.2 ± 0.6	4.1 ± 0.9	4.4 ± 0.8
HDL-C, mmol/l	1.2 ± 0.3	0.6 ± 0.2[a]	0.5 ± 0.2[a, **]
LDL-C, mmol/l	2.9 ± 0.6	2.9 ± 0.9	3.0 ± 0.8
ApoA-I, mg/dl	167.4 ± 9.9	149.6 ± 22.2[a]	130.1 ± 16.5[a, ***]
ApoB, mg/dl	57.9 ± 9.8	83.6 ± 16.4[a]	93.7 ± 14.6[a, **]
ApoB/ApoA-I ratio	0.35 ± 0.06	0.57 ± 0.15[a]	0.74 ± 0.17[a, ***]
CRP, mg/l	3.1 ± 0.9	4.6 ± 0.7[a]	5.7 ± 0.8[a, ***]
TNF-α, pg/ml	3.7 ± 1.1	7.0 ± 1.1[a]	8.5 ± 1.0[a, ***]
IL-6, pg/ml	3.0 ± 0.9	5.1 ± 1.0[a]	6.5 ± 0.8[a, ***]
Resistin, ng/ml	7.7 ± 0.6	8.6 ± 0.7[a]	10.2 ± 0.6[a, ***]

CO = control; Ob = obese; Ob-MS = obese with metabolic syndrome; ApoA-I = apolipoproteinA-I; ApoB = apolipoproteinB, TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin-6; CRP = C-reactive protein; BP = blood pressure; HOMA-IR = homeostasis model assessment-insulin resistance.

*p < 0.05. **p < 0.01. ***p < 0.001 different to Ob. [a]p < 0.001: different to CO.

In Ob, plasma resistin showed significant correlations with insulin, systolic and diastolic BP, TNF-α, and CRP. Additionally, resistin correlated significantly with hip circumference, triglycerides, and HDL-C.

In CO, plasma resistin showed significant correlation only with diastolic BP and IL-6.

Multiple Regression Analyses with Resistin and HOMA-IR as Dependent Variables

Multiple linear regression models, with plasma resistin or HOMA-IR as independent variables including each variable in the different models, are presented in table 3. In Ob-MS, when the analysis was performed with resistin as the dependent variable, systolic BP was the significant determinant. According to the analysis with HOMA-IR as dependent variable, plasma resistin was the significant determinant.

In Ob, plasma triglycerides was the significant determinate of plasma resistin, and HDL-C was the significant determinant of HOMA-IR.

In CO, diastolic BP was the significant determinate of resistin, and ApoB/ApoA-I ratio was the significant determinate of HOMA-IR.

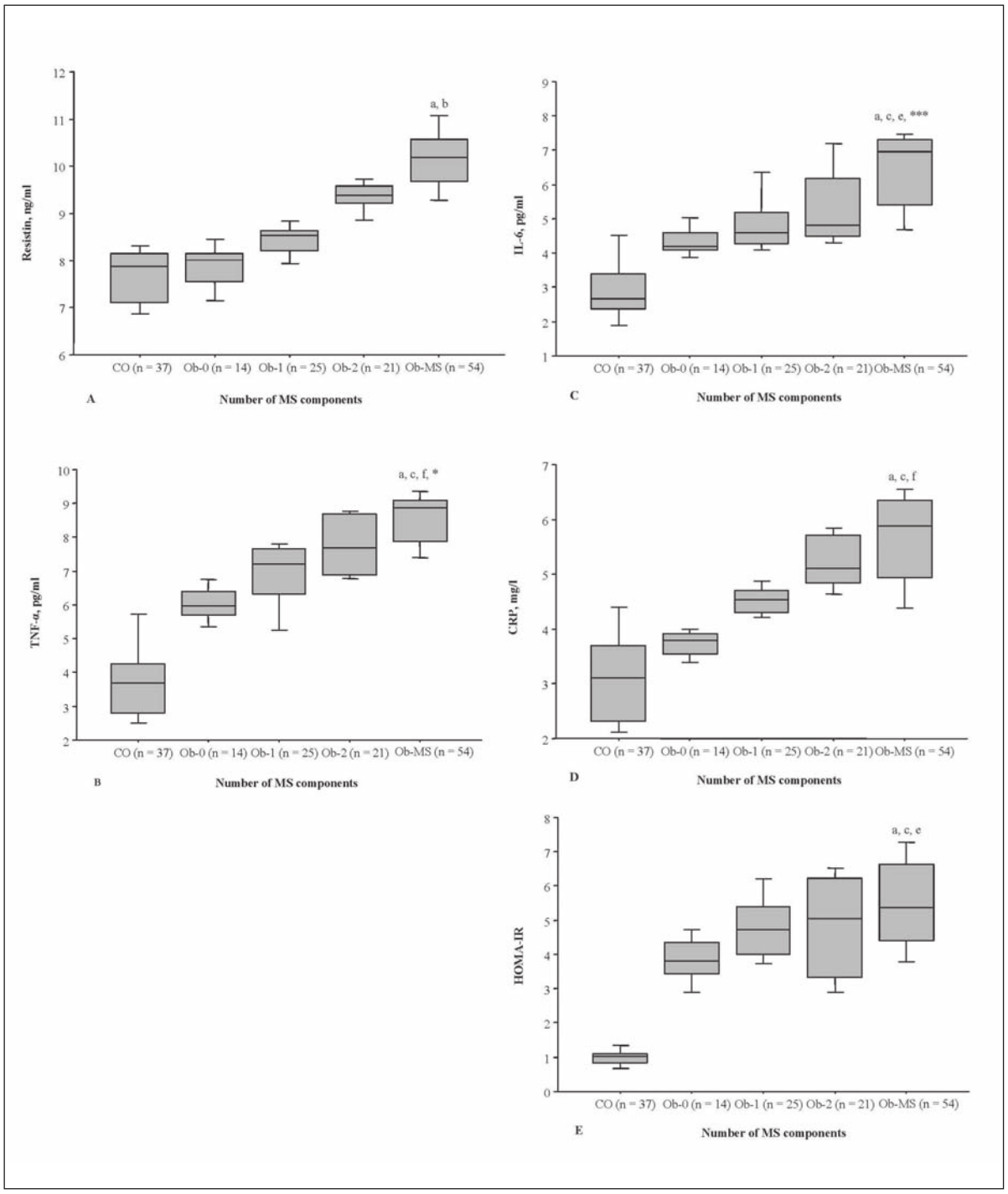

Fig. 1. The comparison of mean values for **A** resistin, **B** TNF-α, **C** IL-6, **D** CRP, and **E** HOMA-IR according to the number of MS components. [a]p < 0.001: different to CO, [b]p < 0.001 different to Ob-0, Ob-1 and Ob-2, [c]p < 0.001 different to Ob-0, [e]p < 0.05 different to Ob-1, [f]p < 0.001 different to Ob-1, *p < 0.05 different to Ob-2, ***p < 0.001 different to Ob-2.

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Table 2. Spearman regression between resistin and anthropometric, blood pressure, and biological variables

Measures	Spearman's regression for resistin					
	CO (n = 37)		Ob (n = 60)		Ob-MS (n = 54)	
	r	p value	r	p value	r	p value
BMI z-score	0.01		0.26	<0.05	0.74	<0.001
Waist circumference	0.05		0.27	<0.05	0.67	<0.001
Hip circumference	0.19		0.28	<0.05	0.60	<0.001
Waist/hip ratio	0.16		0.01		0.28	<0.05
Waist/height ratio	0.15		0.23		0.69	<0.001
Systolic BP	0.15		0.34	<0.01	0.80	<0.001
Diastolic BP	0.46	<0.01	0.34	<0.01	0.72	<0.001
Glucose	0.17		0.18		0.08	
Insulin	0.13		0.32	<0.05	0.72	<0.001
Triglycerides	0.04		0.48	<0.01	0.17	
Total cholesterol	0.15		0.02		0.32	<0.05
HDL-C	–0.10		–0.33	<0.05	–0.27	<0.05
Apo-A-I	0.03		–0.22		–0.50	<0.001
Apo-B	0.01		0.19		0.27	<0.05
Apo-B/ApoA-I ratio	0.02		0.26	<0.05	0.43	<0.01
CRP	0.11		0.61	<0.001	0.65	<0.001
TNF-α	0.05		0.42	<0.01	0.64	<0.001
IL-6	0.36	<0.05	0.20		0.54	<0.001
HOMA-IR	0.02		0.19		0.46	<0.01

HOMA-IR = Homeostasis model assessment-insulin resistance; Ob = obese; Ob-MS = obese with metabolic syndrome; CO = controls; HDL-C = high-density lipoprotein-cholesterol; ApoB/ApoA-I ratio = apolipoproteinB/apolipoproteinA-I ratio; BP = blood pressure; TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin-6; CRP = C-reactive protein.

Table 3. Multiple regression analyses with resistin and HOMA-IR as the dependent variable in the model

Independent variables	Regression analyses											
	resistin						HOMA-IR					
	CO (n = 37)		Ob (n = 60)		Ob-MS (n = 54)		CO (n = 37)		Ob (n = 60)		Ob-MS (n = 54)	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
BMI z-score	0.08		0.05		0.03		0.01		0.15		0.01	
Waist circumference	0.59		0.30		0.20		0.26		0.19		0.17	
Waist/height ratio	0.61		0.11		0.21		0.47		0.30		0.07	
Triglyceride	0.12		0.47	<0.01	0.09		0.01		0.13		0.23	
HDL-C	–0.19		–0.20		–0.15		–0.12		–0.27	<0.05	–0.18	
ApoB/ApoA-I ratio	0.25		0.15		0.07		0.40	<0.05	0.20		0.14	
Systolic BP	0.04		0.31		0.63	<0.01	0.13		0.21		0.10	
Diastolic BP	0.53	<0.01	0.03		0.21		0.23		0.03		0.03	
Resistin							0.05		0.18		0.40	<0.05
HOMA-IR	0.05		0.16		0.19							
R	0.56		0.67		0.84		0.55		0.61		0.61	

HOMA-IR = Homeostasis model assessment-insulin resistance; CO = control; Ob = obese; Ob-MS = obese with metabolic syndrome; HDL-C = high-density lipoprotein-cholesterol; ApoB/ApoA-I = apolipoproteinB/apolipoproteinA-I ratio; BP = blood pressure.

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Table 4. Comparison between low and high resistin level and both anthropometric and biochemical markers

	Resistin cut-off		p value
	<9	>9	
Total number (%)	80 (53)	71 (47)	
CO/Ob/Ob-MS	37/43/0	0/17/54	
Resistin, ng/ml	8.0 ± 0.6	10.1 ± 0.6	<0.001
Gender male, n (%)	39 (49)	37 (52)	0.68
Physical characteristics			
BMI, kg/m^2	25.3 ± 2.8	31.7 ± 4.6	<0.001
BMI z-score	2.5 ± 0.6	3.9 ± 1.1	<0.001
Hip circumference, cm	89.7 ± 16.5	112.5 ± 9.1	<0.001
Metabolic syndrome criteria			
Waist circumference, cm	80.9 ± 14.7	105.1 ± 10.8	<0.001
Systolic BP, mm Hg	123.9 ± 6.1	134.9 ± 5.2	<0.001
Diastolic BP, mm Hg	71.1 ± 7.9	81.8 ± 4.8	<0.001
Glucose, mmol/l	4.0 ± 1.3	4.9 ± 0.9	<0.001
Triglycerides, mmol/l	1.1 ± 0.4	1.7 ± 2.2	<0.001
HDL-C, mmol/l	0.9 ± 0.4	0.5 ± 0.2	<0.001
Inflammatory markers			
CRP, mg/l	3.8 ± 1.0	5.5 ± 0.8	<0.001
TNF-α, pg/ml	5.3 ± 1.8	8.3 ± 1.1	<0.001
IL-6, pg/ml	4.0 ± 1.5	6.2 ± 1.3	<0.001
HOMA-IR	2.8 ± 1.9	5.4 ± 1.4	<0.001

CO = Control; Ob = obese; Ob-MS = obese with metabolic syndrome; TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin-6; CRP = C-reactive protein; BP = blood pressure; HOMA-IR = homeostasis model assessment-insulin resistance.

To understand which factors may influence resistin concentration, we split the subjects according to the 90th percentiles of resistin level (90th percentile > 9 ng/ml) and compared subjects' characteristics and biomarker concentrations according to this cut-off. Results are presented in table 4. Using cut-off analysis, anthropometrics, MS criteria, inflammatory markers, and HOMA-IR were higher in the high-resistin group, even after adjusting for age, sex and pubertal stage. Standard multiple regression analysis including all MS criteria, inflammatory markers, and HOMA-IR showed that in the high-resistin group, systolic BP, IL-6, CRP, and HOMA-IR remained significantly associated with resistin level.

Discussion

The aim of the present study was to examine plasma resistin levels and their correlations with insulin resistance assessed by HOMA-IR and plasma markers of inflammation in both Ob and Ob-MS compared to healthy age-matched CO. The main findings of the present study support a link between circulating resistin and obesity-related inflammatory and cardiovascular events in adolescents and outline evidence for a role of resistin in human childhood MS.

As expected, our results showed that Ob-MS had significantly higher metabolic and cardiovascular risk markers, such as waist circumference, waist/hip and waist/height ratios, triglycerides, insulin, ApoB/ApoA-I ratio and systolic and diastolic BP, than Ob and CO. These results corroborated previous clinical investigations [32–34]. Interestingly, adolescents who combine the highest number of MS components are those with the highest plasma levels of resistin. Furthermore, plasma resistin correlated strongly with many obesity markers, inde-

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DOI: 10.1159/000354574

pendently of age, sex and puberty, such as BMI z-score, waist circumference, and waist/height ratio. In fact, these factors are stronger in Ob-MS than in Ob and disappeared in CO. The association between central obesity and plasma resistin which is supported by the strong linear correlation between plasma resistin and the markers BMI z-score, waist circumference as well as waist/height ratio are in agreement with several previous investigations in children and adolescents [19, 35]. However, the absence of this interdependence between resistin level and anthropometric parameters of central obesity in healthy subjects probably indicates that the relationship found in Ob and Ob-MS subjects is rather related, at least in part, to other abnormal variables found in this population than to a direct effect of adipose tissue. Accordingly, standard multiple regression analysis with plasma resistin and HOMA-IR as the dependent variables including each variable in the opposite models highlighted that plasma resistin affected HOMA-IR and was the only significant predictor of HOMA-IR in Ob-MS. As the standard multiple regression models showed that the degree of adiposity was not related to the production of resistin, we investigated the raw resistin values and factors associated with increased resistin levels. According to Magio et al. [36], we chose a cut-off value of 9 ng/ml, which was the 90th percentile of resistin levels in our population. In our study, using the multiple regression analysis, high resistin level (>9 ng/ml) was not related to anthropometric makers of central obesity but to biochemical markers of inflammation. In fact, IL-6 was the main cytokine related to resistin level. This link between IL-6 and resistin is still poorly understood, but an in vitro study showed that the production of IL-6 and other cytokines such as IL-1 and TNF-α increases the expression of resistin in mononuclear cells [23], and others confirm this role for TNF-α, but not for IL-6 or IL-1. However, Suliga [37] showed that the increase of resistin stimulates the secretion of the 3 cytokines, suggesting that resistin exerts a control of the cytokine inflammatory cascade.

Resistin is an adipocyte-specific protein that plays a role in metabolic and cardiovascular disease-related obesity. However, the relationship between circulating resistin and insulin resistance in obese populations is still controversial [15–19]. In adults, several studies have reported an association between these factors [18, 19], whereas other authors failed to find any significant correlation [18, 19]. Plasma resistin was found by some investigators to be increased in obesity and to be positively correlated with insulin resistance [17, 18] while this observation could not be confirmed in children and adolescents [19, 35]. The results of the present study support the hypothesis of an association between circulating resistin and insulin resistance, confirming previous findings in adults [17, 18]. However, it has been suggested that plasma resistin was not an independent predictor of an obese and insulin-resistant phenotype in Chinese children and adolescents [19]. There was also evidence that plasma resistin levels varied according to age, sex, and puberty stage. In our study, correlation analyses were adjusted for these confounding factors. In this context, we have not investigated if there are any effects related to sex on plasma resistin levels since the main purpose of the current study was to examine the relationships between resistin and cardiovascular diseases, inflammation as well as metabolic disorders related to childhood obesity. On the other hand, we have compared boys and girls (unpublished data), and no significant differences between sexes have been observed. The discrepancies in the results of our and other studies [19, 35] with respect to a relationship between plasma resistin levels and obesity or insulin resistance in children or adolescents are probably due to genetic and ethnical differences [38]. In fact, it has been reported that genetic polymorphisms in the promoter region of the resistin gene may be independent predictors of circulating resistin concentrations in humans [39, 40].

An important recent development in our understanding of obesity pathophysiology was the emergence of the concept that, along with diabetes, this condition is characterized by a state of chronic, low-grade inflammation [41]. Similar to many studies in children and adoles-

cents [19, 35], we also found that circulating levels of TNF-α, IL-6, and CRP increased depending on the severity of MS, with highest values in Ob-MS. Interestingly, in Ob-MS with low-grade inflammation a significant correlation between these pro-inflammatory markers and plasma resistin was found, and the plasma resistin level itself correlated well with obesity markers, suggesting that the effects of pro-inflammatory molecules on resistin levels were dependent on obesity. On the other hand, the strong correlation of resistin with central obesity, which is associated with inflammation [19, 35, 42], suggested that increased plasma resistin observed in children with central obesity was linked to a pro-inflammatory state. Moreover, in both linear and multiple regression models the systolic BP was significantly correlated with plasma resistin levels in Ob-MS. This finding corroborate the data of Roth et al. [35] who had found that changes of resistin were correlated positively with changes of systolic BP ($r = 0.20$, $p = 0.03$). Similarly, Li et al. [19] demonstrated that diastolic and systolic BP were correlated positively with resistin in boys, even though no such correlation was observed in girls. The resistin effects on BP may be explained by a resistin-induced increase in mRNA abundance of fatty acid binding protein in human coronary artery endothelial cells. Other potential mechanisms linking resistin to hypertension include an ability to promote smooth muscle cell proliferation [43] and vasoconstrictor properties of resistin [44].

There are also some potential limitations in the design of our study that should be mentioned. Firstly, it would be interesting to extend this analysis on a larger population to confirm and reinforce that there is a relationship between plasma resistin and insulin resistance as well as MS in obese children and adolescents. Secondly, the HOMA-IR model is only an assessment of insulin resistance. Clamp studies are currently the gold standard for analyzing insulin resistance [45]. Finally, it would be interesting to investigate the genetic factors responsible for the variation of plasma resistin levels according to sex and MS components in this youth population. This will allow us to analyze the mechanism of action of resistin on childhood obesity-related cardiometabolic events and insulin resistance.

Conclusion

The present study demonstrated that plasma resistin showed higher correlations with anthropometric parameters, lipid profiles, systolic and diastolic BP and pro-inflammatory cytokines in Ob-MS. Additionally, the multiple regression models highlighted that plasma resistin is related to systolic BP, IL-6, and HOMA-IR in the high-resistin level group. These results support the hypothesis that there is an association between circulating resistin and obesity-related inflammatory as well as cardiometabolic events in humans' childhood obesity.

Acknowledgements

This study was supported by the Ministry of Higher Education and Scientific Research of Tunisia. The authors would like to thank the participating children and their parents for their strong cooperation and availability.

Disclosure Statement

No conflict of interest is declared.

Obes Facts 2013;6:393–404
DOI: 10.1159/000354574

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