

Relationship between Resistin, Endothelin-1, and Flow-Mediated Dilation in Patient with and without Metabolic Syndrome

Abstract

Background: Resistin is peptides that signal the functional status of adipose tissue to the brain and other target organs. It causes insulin resistance and affects the vascular endothelial dysfunction. However, the function and relation between resistin in endothelin-1 (ET-1), which leads to the endothelial dysfunction in humans are enigmatic. **Materials and Methods:** In a cross-sectional study of 76 participants (38 metabolic syndrome patients and 38 healthy participants), biochemical and clinical parameters, including lipid profile, fasting glucose, resistin, ET-1, C-reactive protein (CRP), flow-mediated dilation (FMD), and hypertension were determined and compared between the two groups. **Results:** Multiple linear regression analysis was performed with age- and sex-adjusted plasma resistin levels, FMD, and ET-1 as the dependent variables. Analysis showed that weight, body mass index, triglycerides (TGs), and ET-1 were statistically significant correlated with serum resistin. FMD has negative significantly correlated with weight ($r = -0.491$, $P = 0.001$), waist circumference ($r = -0.491$, $P = 0.001$), waist-to-hip ratio ($r = -0.0444$, $P = 0.001$), and ET-1 ($r = -0.075$, $P = 0.050$), but it has significantly correlated with systolic blood pressure (SBP) ($r = 0.290$, $P = 0.016$), diastolic blood pressure (DBP) ($r = 0.275$, $P = 0.023$), and high-density lipoprotein cholesterol (HDL-C) ($r = -0.266$, $P = 0.050$), and ET-1, but it has significantly correlated with SBP, DBP, and HDL-C. ET-1 is significantly correlated with TGs ($r = -0.436$, $P = 0.006$), total cholesterol ($r = 0.452$, $P = 0.004$), low-density lipoprotein cholesterol ($r = 0.454$, $r = 0.004$), and resistin ($r = 0.282$, $P = 0.050$), whereas it has negative significantly correlated with HDL-C ($r = 0.346$, $P = 0.034$), FMD ($r = -0.075$, $P = 0.050$). **Conclusion:** In this study, results shown plasma ET-1 and resistin are suggested as risk factors for the development of endothelial dysfunction and with further study, it is possible that can diagnose the risk of diabetes and cardiovascular disease in the early stages.

Keywords: Endothelin-1, flow-mediated dilation, metabolic syndrome, resistin

Introduction

Metabolic syndrome (MetS), is the compilation of risk factors that include hypertension, dyslipidemia, insulin resistance, and hyperglycemia, is the public health problem that significantly contributes to the development of cardiovascular disease (CVD).^[1,2]

Visceral adipose tissue plays a key role in the pathogenesis of MetS and its complications.^[3] Several adipokines have been illustrated to exert the regulatory roles on CVD risk factors, for example, inflammation, adipogenesis, lipid metabolism, and oxidative stress.^[4-6] Resistin is an adipocyte- and monocyte-derived cytokine which modulates insulin action, energy, and glucose and lipid homeostasis.^[7]

In humans, resistin is predominantly produced by peripheral blood mononuclear

cells (PBMCs), macrophages, and bone marrow cells.^[8-10]

It has been proposed that resistin might serve as a molecular link between the inflammation, metabolic parameters, and vascular dysfunction, and can thus contribute to the risk for MetS, Type 2 diabetes mellitus (T2DM), and CVD.^[11,12] However, human studies shows controversial role of resistin in regulating insulin sensitivity and obesity.^[13-15]

Endothelin-1 (ET-1) is a powerful endogenous vasoconstrictor peptide that is produced and released by the vascular endothelium.^[16] Moreover, it has been linked to the pathogenesis of hypertension, heart failure, and atherosclerotic vascular disease.^[17,18] Weil *et al.* have previously reported that ET-1 vasoconstrictor tone is elevated with increases in adiposity,

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contributing to diminished endothelium-dependent vasodilation, and augmented cardiovascular risk in overweight and obese adults.^[19]

Previous studies showed that an increase in resistin concentration significantly decreases an endothelial nitric oxide (NO) synthase expression and NO production through oxidative stress in cultured human coronary artery endothelial cells.^[20]

Flow-mediated dilation (FMD) refers to the dilation of an artery when blood flow increases in that artery. The primary cause of FMD is a release of NO by the endothelial cells. Endothelial function is measured *in vivo* through measuring FMD in the brachial artery. FMD has been proven to be a strong predictor of cardiovascular events.^[21]

Therefore, the present study aimed to compare the relationship between resistin, ET-1, and FMD in patients with and without MetS.

Materials and Methods

Subjects and design

A total of 76 participants were chosen, from those who referred to the Isfahan Cardiovascular Research Center. Participant's eligibility was determined using a modified version of the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII). Criteria for MetS, were according to the published criteria.^[22] Participants were required to meet at least three of the following five criteria: (1) (a) Abdominal obesity, defined as waist circumference >102 cm for men or >88 cm for women; (b) elevated serum triglyceride (TG) (≥ 150 mg/dL); (c) low-serum high-density lipoprotein cholesterol (HDL-C (<40 mg/dL for men and <50 mg/dL for women), (d) hypertension (blood pressure [BP] $\geq 130/85$ mmHg) or current treatment for hypertension, and (e) impaired fasting plasma glucose (FPG) ≥ 110 mg/dL); (2) Age >18 years; (3) Free of diseases affecting serum lipids (e.g. thyroid disorders and pancreatitis); (4) Free of liver or kidney disease; (5) Not being substance abuser (including alcohol) or smoker; and (6) Not being pregnant or lactating (for women). The healthy participants age >18 years, free of hyperlipidemia, thyroid disorders, liver or kidney disease, and nonsmoker were chosen.

The participants in this cross-sectional study were 72 adult individuals and 38 with and 38 without MetS. The study protocol was approved by the Medical Ethics Committee of the Isfahan Cardiovascular Research Institute under the Approval No. 91115.

Anthropometric measurements

All participants were evaluated for weight (Wt.), height (Ht.), body mass index (BMI), waist circumference, and waist-hip ratio (WHR) (a good marker for measuring

central/visceral obesity). Weight was measured to the nearest 0.1 kg with calibrated digital scales (AMZ 14; Mercury, Tokyo, Japan). Stature and circumference measurements were made to the nearest 0.1 cm standing height was measured with a wall-mounted stadiometer. BMI was calculated by dividing the weight (in kilograms) by height (in meters) squared. Waist circumference was measured with a tape measure at the uppermost lateral border of the hip crest (ilium).^[23]

Biochemical determinations

Blood samples

Blood samples obtained by venipuncture after a 12–14 h fasting. Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) to yield a final concentration of 0.1% EDTA. Plasma was separated from red cells by centrifugation at $1500 \times g$ for 15 min at 4°C within 1 h of extraction.^[24]

Biochemical analysis

Fresh serum samples were used to measure FBG, total cholesterol (TC), TG, low-density lipoprotein cholesterol (LDL-C), and HDL-C. Enzymatic methods with commercial kits were used for the measurement of lipid profile parameters in all the participants.

Plasma samples were kept on dry ice during transportation from the testing sites and were stored at -80°C until analyses, to measure resistin and ET-1.

Plasma resistin was quantitated using an enzyme-linked immunosorbent assay kit obtained from a commercial kit (Phoenix Pharmaceuticals Inc., USA). The lower detection limit of the assay was 0.0625 ng/mL. Intra- and inter-assay coefficients of variation for the assay were 3% and 10%, respectively.^[24]

For plasma ET-1, 10 mL of venous blood was collected into an EDTA tube and for 20 min at 4°C centrifuged immediately at 2500 g. ET-1 was measured by commercially available ELISA kits (Morinaga and R and D System).^[25] Standards, reagents, and test samples were prepared and assayed according to the instructions of the manufacturer.

Flow-mediated dilation measurements

FMD was measured by ultrasonography with an automated edge tracking system (UNEX 18G, UNEX Co. Nagoya, Japan) as previously described.^[26] The vascular response to 5 min reactive hyperemia in the brachial artery was used for the assessment of endothelium-dependent FMD.

Statistical analysis

All the clinical data and anthropometric values are presented as mean \pm standard deviation. Statistical analysis was conducted by using SPSS Version 15.0 for Windows (SPSS 20; IBM SPSS, Chicago, IL, USA). Pearson's

correlation coefficient was performed to determine the relation between plasma resistin levels, FMD, ET-1, and the other continuous variables. Differences between the groups of all measurements were calculated using the independent samples *t*-test. Multiple linear regression analysis was performed with age and sex-adjusted plasma resistin levels, FMD, and ET-1 as the dependent variables. For all analysis, *P* < 0.05 was considered as statistically significant.

Results

Anthropometric and cardiac variables as well as biochemical parameters of the study participants are summarized in Table 1. In MetS group, all data were significantly higher than in the normal group (except FMD and HDL-C that less than in the normal group) (*P* < 0.05). Multiple linear regression analysis was performed with age and sex-adjusted plasma resistin levels, FMD, and ET-1 as the dependent variables.

The analysis showed that weight (*r* = 0.396, *P* = 0.014), BMI (*r* = 0.302, *P* = 0.050), TGs, (*r* = 0.384, *P* = 0.023) and ET-1 (*r* = 0.282, *P* = 0.050) were statistically significant correlated with serum resistin [Table 2].

FMD has negative significantly correlated with weight (*r* = -0.491, *P* = 0.001), waist circumference (*r* = -0.491, *P* = 0.001), WHR (*r* = -0.0444, *P* = 0.001), and ET-1 (*r* = -0.075, *P* = 0.050), but it has significantly correlated with systolic BP (SBP) (*r* = 0.290, *P* = 0.016), diastolic BP (DBP) (*r* = 0.275, *P* = 0.023), and HDL-C (*r* = -0.266, *P* = 0.050) [Table 3].

ET-1 is statistically significant correlated with TGs (*r* = -0.436, *P* = 0.006), TC (*r* = 0.452, *P* = 0.004), LDL-C (*r* = 0.454, *r* = 0.004), and resistin (*r* = 0.282, *P* = 0.050), whereas it has negative significantly correlated with HDL-C (*r* = 0.346, *P* = 0.034), FMD (*r* = -0.075, *P* = 0.050 [Table 4].

Discussion

Investigation showed that anthropometric and cardiac variables as well as inflammatory and biochemical parameters are significantly higher in MetS compared with the controls, whereas FMD levels and HDL-C were found significantly lower in MetS participants compared with the controls.

The main finding of the present cross-sectional study is that ET-1 and resistin can be considered as a candidate marker for cardiovascular risk. Because resistin has significant associations with endothelial biomarker (ET-1) and reverse relationship with FMD in MetS participant.

Previous studies showed that an increase in resistin concentration significantly decreases the endothelial NO synthase expression and NO production through oxidative stress in cultured human coronary artery endothelial

Table 1: Characteristics of the study population (38 subjects in each group) addjusted for age and sex

parameters	Group normal	Group metabolic syndrome	<i>p</i>
Anthropometric parameters			
Age (years)	44.24±10.55	44.21±11.06	>0.001
BMI(kg/m)	22.26±4.33	28.89±4.78	<0.001
Waist circumference (cm)	85.76±10.20	101.42±9.48	<0.001
Hip circumference, (cm)	100.45±6.09	111.16±8.67	<0.001
WHR	0.88±0.07	0.92±0.05	<0.001
Cardiac variables			
SBP (mm Hg)	106.89±9.62	123.92±13.43	<0.001
DBP (mm Hg)	68.66±6.94	77.76±9.56	<0.001
FMD (%)	4.17±0.69	3.84±0.55	0.024
Biomedical parameters			
FBG (mmol/L)	82.39±6.04	96.26±18.53	<0.001
Triglycerides, mmol/l	113.71±32.29	208.47±69.29	<0.001
Total cholesterol, mmol/l	176.03±40.32	208.58±41.47	<0.001
HDL-C, mmol/l	44.89±7.51	38.45±6.36	<0.001
LDL-C, mmol/l	89.24±23.92	97.82±22.51	<0.001
CRP, mg/l	3.02±1.45	4.65±3.01	<0.001
Resistin (ng/ml)	0.82±0.44	1.70±0.54	0.004
ET-1(pg/ml)	71.44±132.82	145.73±223.03	0.008

FMD: Flow-mediated dilation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HDL: High density lipoprotein; LDL: Low density lipoprotein; CRP: C-Reactive protein; ET-1: Endothelin-1

Table 2: Linear regression analysis for the correlation of Resistin and risk factors

Resistin	Group normal		Group metabolic syndrome	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Weight(kg)	-0.096	0.566	-0.396	0.014
BMI(kg/m)	-0.031	0.872	-0.302	0.050
Waist circumference (cm)	-0.291	0.158	-0.222	0.216
WHR	0.278	0.160	0.013	0.951
SBP (mm Hg)	-0.070	0.676	-0.128	0.452
DBP (mm Hg)	-0.226	0.171	-0.021	0.909
FMD	-0.297	0.192	-0.002	0.990
FBG (mmol/L)	-0.006	0.975	-0.097	0.576
Triglycerides, mmol/l	-0.001	0.996	-0.384	0.023
Total cholesterol, mmol/l	-0.090	0.600	-0.058	0.728
HDL-C, mmol/l	0.180	0.341	-0.098	0.643
LDL-C, mmol/l	-0.068	0.684	-0.059	0.32
CRP, mg/l	-0.043	0.797	-0.068	0.695
ET-1(pg/ml)	0.009	0.956	0.282	0.050

FMD: Flow-mediated dilation; BMI: body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HDL: High density lipoprotein; LDL: Low density lipoprotein; CRP: C-reactive protein; ET-1: Endothelin-1

Table 3: Linear regression analysis for the correlation of flowmediated dilation (FMD) and risk factors

FMD	Group normal		Group metabolic syndrome	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Weight(kg)	-0.293	0.056	-0.491	0.001
BMI(kg/m)	0.207	0.152	0.247	0.061
Waist circumference (cm)	-0.293	0.056	-0.491	0.001
WHR	-0.039	0.833	-0.444	0.001
SBP (mm Hg)	0.211	0.147	0.290	0.016
DBP (mm Hg)	0.252	0.099	0.275	0.023
FBG (mmol/L)	-0.218	0.140	-0.223	0.078
Triglycerides, mmol/l	-0.128	0.407	-0.152	0.243
Total cholesterol, mmol/l	-0.050	0.732	-0.003	0.983
HDL-C, mmol/l	-0.116	0.523	0.266	0.050
LDL-C, mmol/l	-0.192	0.194	-0.046	0.711
CRP, mg/l	-0.030	0.837	-0.001	0.933
Resistin (ng/ml)	0.034	0.818	0.203	0.115
ET-1(pg/ml)	-0.132	0.375	-0.075	0.050

FMD: Flow-mediated dilation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HDL: high density lipoprotein; LDL: Low density lipoprotein; CRP: C-reactive protein; ET-1: Endothelin-1

Table 4: Linear regression analysis for the correlation of endothelin-1 and risk factors

endothelin-1	Group normal		Group metabolic syndrome	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Weight(kg)	-0.095	0.572	-0.008	0.960
BMI(kg/m)	-0.091	0.589	0.085	0.614
Waist circumference (cm)	-0.094	0.574	-0.127	0.447
WHR	-0.258	0.118	-0.079	0.636
SBP (mm Hg)	-0.122	0.465	-0.242	0.144
DBP (mm Hg)	0.019	0.910	-0.242	0.143
FMD	-0.132	0.375	-0.075	0.050
FBG (mmol/L)	-0.209	0.207	-0.012	0.942
Triglycerides, mmol/l	-0.059	0.727	-0.436	0.006
Total cholesterol, mmol/l	0.344	0.350	0.452	0.004
HDL-C, mmol/l	0.027	0.873	0.346	0.034
LDL-C, mmol/l	0.198	0.234	0.454	0.004
CRP, mg/l	-0.074	0.660	0.048	0.777
Resistin (pg/ml)	0.009	0.956	0.282	0.050

FMD: Flow-mediated dilation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HDL: High density lipoprotein; LDL: Low density lipoprotein; CRP: C-reactive protein; ET-1: Endothelin-1

cells.^[20] FMD refers to dilation of an artery when blood flow increases in that artery. The primary cause of FMD is a release of NO by the endothelial cells. Endothelial function is measured *in vivo* through measuring FMD in the brachial artery. FMD has been proven to be a strong predictor of cardiovascular events.^[21]

In this study, FMD has positively correlated with SBP, DBP, and HDL-C, also result showed FMD was inversely

associated with serum concentrations of ET-1, waist circumference, and weight. This result is consistent with other studies.^[27,28]

Insulin resistance causes vasoconstriction that with reduced biological activity-associated increased production and secretion of NO and ET-1.^[29] Both ET-1 and NO are produced by endothelial cells, they have opposing functions, the former being a vasoconstrictor and the latter a vasodilator. Therefore, it is important for the vascular pathophysiology of preeclampsia to evaluate the ET-1 and NO metabolites simultaneously. Furthermore, FMD is a noninvasive index of endothelial function and vascular health in humans. Studies revealed that FMD of conduit arteries in humans is, at least in part, mediated by NO.^[30] Studies reported that resistin increases the expression of vasoconstrictor ET-1, which may promote endothelial dysfunction.^[31]

The connection between the MetS and endothelial dysfunction is firmly established; the underlying pathophysiological mechanisms are only partially understood. Although lower FMD has been reported in young males with hypertriglyceridemia,^[32] acute administration of TGs to normal individuals has not been shown to affect endothelial function.^[33]

The present study revealed that ET-1 is significantly correlated with endothelial function, lipid profile, and resistin. The adverse effects of plasma ET-1 on endothelial function have also been found in other recent studies.^[34] Rocha *et al.*, shown that MetS is associated with higher ET-1 vasoconstrictor tone in overweight/obese adults.^[35] Studies reported that resistin increases the expression of vasoconstrictor ET-1, which may promote the endothelial dysfunction.^[31]

The present study revealed that resistin is significantly correlated with the endothelial function. The adverse effects of plasma resistin on endothelial function have also been found in other recent studies.^[34]

Resistin plays an important role in the pathogenesis of MetS and enhanced atherogenesis.^[31,36] The finding of this study is that plasma resistin levels are positive correlated with a weight, BMI, TGs and with ET-1 in the MetS patients. Some human studies reported that plasma resistin levels positively correlated with obesity, insulin resistance, and T2DM,^[11,12,37] while other studies failed to observe any correlation of plasma resistin levels with either metabolic or lipid markers,^[38,39] and no significant difference was observed in plasma resistin levels in participants with the MetS compared to the controls.^[40,41]

A cohort studies revealed that plasma resistin levels were positively correlated with HDL-C, TGs, waist circumference, and SBP insulin, as well as with BMI, furthermore, participants with the MetS showed higher plasma resistin levels compared with the controls.^[37,42]

Asgary *et al.* were found correlation of plasma resistin levels with levels of TC and LDL-C, but not other components of MetS including systolic and DBP, TG, HDL-C, and FBS in the MetS group, after the adjustment for age, gender, and BMI.^[14] Utzschneider *et al.* find any correlation of plasma resistin levels with the MetS.^[40] The serum resistin levels were inversely associated with serum concentrations of HDL-cholesterol which were in agreement with the previous report study.^[43]

In this study, the serum resistin level did not correlate with SBP and DBP. This relationship was also found in healthy participants,^[44] participants with hypertension, and those suffering from T2DM.^[45]

Kunnari *et al.* showed a positive correlation of resistin levels with leukocytes and high-sensitive C-reactive protein (CRP).^[46] Different explanations could account for these discrepancies, including the use of different assay methods, the low number of patients enrolled in the different studies, and the definition used to select patients with the MetS. Despite these findings, the role of resistin in the MetS is controversial.

Conclusion and Limitations

In this study, results shown plasma ET-1 and resistin are suggested as risk factors for the development of endothelial dysfunction and with further study, it is possible that can diagnose the risk of diabetes and cardiovascular disease in the early stages.

Since, both inflammation and insulin resistance play critical roles in the development of atherosclerosis, associations with biomarkers of inflammation and/or endothelial function would imply that resistin could possibly be directly or indirectly related to MetS and CVD.^[47] We did not check the inflammation factor (Except CRP) and insulin resistance, which have been identified as important mediators of endothelial damage. Furthermore, the duration and amount of cigarette smoking were not included in our data. This is another limitation in our study. Furthermore, we did not evaluate the potent effect of physical activity and the nature of the work of the examined participants on the correlation to the examined parameters. It is recommended that these be considered in the future studies.

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Conflicts of interest

There are no conflicts of interest.

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