Circulating resistin protein and mRNA concentrations and clinical severity of coronary artery disease

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Abstract

Introduction: Previous studies have implicated a strong link between circulating plasma resistin and coronary artery disease (CAD). The aim of this study was to evaluate the differences in peripheral blood mononuclear cells (PBMC) resistin mRNA and its plasma protein concentrations between the patients with CAD of different clinical severity.

Material and methods: This study included 33 healthy subjects as the control group (CG) and 77 patients requiring coronary angiography. Of the latter 30 was CAD negative whereas 47 were CAD positive [18 with stable angina pectoris (SAP) and 29 with acute coronary syndrome (ACS)]. Circulating resistin was measured by ELISA; PBMC resistin mRNA was determined by real-time PCR.

Results: Resistin protein was significantly higher in the ACS group compared to the CG ($P = 0.001$) and the CAD negative group ($P = 0.018$). Resistin mRNA expression did not vary across the study groups, despite the positive correlation seen with plasma resistin ($\rho = 0.305$, $P = 0.008$). In patients, plasma resistin and PBMC resistin mRNA negatively correlated with HDL-C ($\rho = -0.404$, $P < 0.001$ and $\rho = -0.257$, $P = 0.032$, respectively). Furthermore, the highest plasma resistin tertile showed the lowest HDL-C ($P = 0.006$). Plasma resistin was positively associated with serum creatinine ($\rho = 0.353$, $P = 0.002$).

Conclusion: Significant increase of plasma resistin in patients with ACS compared to CG and CAD negative patients was observed. Despite no change in PBMC resistin mRNA in different disease conditions a positive association between resistin mRNA and resistin plasma protein was evident. Both plasma resistin and PBMC resistin mRNA were negatively associated with plasma HDL-C, and plasma resistin positively with serum creatinine.

Key words: resistin, human; gene expression; coronary artery disease; acute coronary syndrome

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Introduction

Coronary artery disease (CAD) is one of the major causes of morbidity and loss of quality of life across the globe. The pathogenesis of CAD lays in the development of dyslipidemia, increased proinflammatory processes and consequently atherosclerosis evolvement and progression (1). Complications of CAD vary depending on the extent and perhaps more importantly on the stability of atherosclerotic lesions: from stable conditions, such as stabile angina pectoris (SAP) to acute coronary syndrome (ACS) which includes unstable angina pectoris (UAP) and acute myocardial infarction (AMI) (2).

Resistin, a 12.5 kDa peptide, first discovered in mouse white adipose tissue has been linked to insulin resistance and type 2 diabetes development in mice (3). However, in humans, that link has been by far questioned (4). Human resistin belongs to a family of cysteine-rich secretory proteins that include resistin-like molecules (RELMs) (5). Unlike in mice, where it is expressed predominately by adipocytes (3), human resistin is expressed and secreted mainly by peripheral blood mononuclear cells (PBMCs), macrophages and bone marrow cells (6). Human resistin has been shown to have

proinflammatory properties and it is involved in complex feedback interactions with other proinflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β) (7). Adenylate cyclase-associated protein 1 (CAP1 protein), a recently identified *bona fide* receptor for human resistin, is proposed to conduct these inflammatory signals, through activation of adenylate cyclase, and increased production of cyclic adenosine monophosphate, which activates protein kinase A and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways leading to increased production of IL-6, TNF-α and IL-1β (8).

Resistin exerts many effects contributing to atherosclerosis. *In vitro* experiments have shown that resistin leads to decreased expression of nitric oxide synthase in human coronary artery endothelial lines (HCAECL) (6), increases monolayer permeability of HCAECLs (9), accelerates plaque progression by stimulating monocyte infiltration (10), upregulates endothelin-1, vascular cell adhesion molecule -1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein (MCP-1) in vascular endothelial cells (6) and increases lipid accumulation in macrophages contributing to foam cell formation (6,11).

Data from clinical studies have revealed an association between resistin protein and traditional risk factors for atherosclerosis: Inverse correlations were observed with protective high-density lipoprotein cholesterol (HDL-C) and total cholesterol (TC); positive correlations were observed with triglycerides (Tg), high sensitivity C-reactive protein (hsCRP) and homeostasis model assessment of insulin resistance (HOMA-IR) (12,13). Plasma resistin has also been shown to be positively correlated with creatinine and was dependent on kidney function: Elevated resistin concentrations in chronic kidney disease were associated with decreased glomerular filtration rate (GFR) (14).

A strong link between circulating plasma resistin and both the presence and severity of CAD is known (6,15), although in some studies the link was lost after adjustment for traditional risk factors (12). Furthermore, resistin concentrations increase with symptomatic severity of CAD (6,16,17), indicating that increased resistin concentrations may be a marker of myocardial ischemia and injury in ACS (6). Plasma resistin has been shown to be positively correlated with PBMC resistin mRNA in type 2 diabetes patients (18). However, the expression of resistin mRNA has not been described in CAD patients.

As PBMCs are one of the main sources of circulating plasma resistin, we sought to investigate whether PBMC resistin mRNA and its plasma protein concentration were changed in patients with presenting different CAD symptoms.

Material and methods

Subjects

This study was designed as an observational, case-controlled study. Patients from the Institute for Cardiovascular Diseases at the Clinical Center of Serbia (Belgrade) who were admitted from February 2012 to January 2013 with requirements for coronary angiography were included in this study (77 patients, 38 male, 39 female). Indications for coronary angiography were stable angina; unstable angina; post-ischemic revascularization; AMI stratification post-AMI; undetermined chest pain origin; heart failure; valvulopathy; non-specific symptoms (asthenia, syncope and dyspnea); presurgery for non-cardiac disease (19-21). The presence or absence of CAD was assessed using maximal luminal narrowing estimated by visual analysis using angiography. Clinically relevant CAD was considered when there was more than 50% stenosis in at least one major coronary artery (22). There were 30 patients without any evidence of CAD (CAD negative) and 47 CAD patients. 18 CAD patients were diagnosed with SAP. 29 CAD patients were diagnosed as having ACS including UAP and AMI diagnosed two weeks before blood was drawn. Criteria for SAP patients were the following: a three month history of chronic, stable, effort-induced angina relieved by rest or nitroglycerine; during exercise tests (experience of limiting angina and ST-segment depression of 0.1 mV compared with rest (19). If patients had chest pain with

elevated troponin concentrations with or without ST-segment elevation seen on electrocardiogram (ECG) they were diagnosed as having AMI. Unstable angina (UA) was diagnosed in patients who were admitted with worsening angina, acute angina after exercise within 3 weeks or renewed angina at rest within 3 weeks (all without any evidence of myocardial infarction as confirmed by subsequent serial ECG and myocardial biomarkers (20,21). Patients with diabetes mellitus (type I or II) and chronic renal failure were excluded from this study. Demographic data including age, body mass index (BMI), as well as data regarding medication used by patients were collected by a questionnaire.

The CG in this study consisted of 33 healthy blood donors (15 males and 18 females), who were selected after thorough assessment of their health status, intima media thickness (IMT) measurement of both carotid arteries and review of their medical history to determine the presence or absence of cardiovascular disease. Demographic data including age, BMI, as well as data regarding medication used by healthy blood donors, and family history indicating possible presence of absence of cardiovascular disease were collected by a questionnaire. Lipid profile was evaluated after the measurement of lipid parameters. Exclusion criteria for the CG were the presence of cardiovascular disease in family history, > 1 mm IMT in at least one carotid artery; hypertension (diastolic blood pressure ≥ 90mm Hg and/or systolic blood pressure ≥ 140mm Hg) or use of any antihypertensive medication; unfavorable lipid profile (according to Adult Treatment Panel III guidelines – ATP III (23): TC > 5.16 mmol/L, LDL-C > 3.35 mmol/L, HDL-C < 1.03 mmol/L, Tg > 1.70 mmol/L) or use of lipid lowering medication; presence of diabetes, glucose ≥ 6.1 mmol/L, presence of obesity (BMI > 30 kg/m^2).

Along with plasma resistin and PBMC resistin mRNA in all subjects creatinine, lipid parameters (TC, HDL-C, LDL-C, Tg) and hsCRP were measured in order to assess their kidney function, lipid status and if low grade inflammation was present. Insulin resistance was evaluated by measuring glucose and insulin concentrations and calculation of the HOMA-IR index. Additionally, considering resistin's connection to proinflammatory cytokine IL-6, plasma IL-6 as well as PBMC IL-6 mRNA expression was also determined.

This study was conducted according to the guidelines laid down in Declaration of Helsinki and all procedures involving human subjects were approved by the ethical committee of Faculty of Pharmacy, University of Belgrade (approval number: 797/2) and School of Medicine, University of Belgrade (approval number: 29/11-15). All participants signed an informed consent before the enrolment.

Methods

Twelve hour fasting whole blood was drawn from all subjects, between 7 and 8 am. Blood was collected into one serum and one EDTA containing tubes (BD Vacutainer®, New Jersey, USA). Serum and plasma were isolated after a 10 minute centrifugation at 1000xg. Serum was analysed immediately after separation, while plasma was stored at -80°C for up to one year until analysis. PBMCs were immediately collected using Ficoll-Paque™ PLUS (GE Healthcare, Waukesha, Wisconsin, USA) reagent according to the manufacturer's protocol. Collected PBMCs were resuspended in TRIzol™ reagent (Ambion, Life technologies, Grand Island, New York), and total RNA was isolated according to the manufacturer's protocol.

The quantity of isolated RNA was assessed by measuring absorbance at 260 nm, while quality was determined by measuring absorbance ratios at 260 nm and 280 nm (A260/280) as well as 260 nm and 230 nm (A260/230), to estimate protein and organic impurity, respectively. Samples with A260/280 ratio greater than 1.8 and a A260/230 ratio greater than 2.4 were used. All absorbance measurements were performed on a Shimadzu 1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). RNA integrity was evaluated by horizontal submarine electrophoresis (Hoefer, Inc. Holliston, Massachusetts, USA). The 18S and 28S ribosomal RNA (rRNA) bands were visualised with ethidium bromide on a MacroVue UV-25 trans-illuminator (Hoefer, Inc. Holliston, Massachusetts, USA) at 306 nm. Samples with clearly visible both subunits were used.

All extracted RNAs were stored at -80 °C prior to reverse transcription (RT) reactions for up to one year. RT and real-time PCR experiments were carried out on the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using TaqMan® reagent-based chemistry. RT was performed using high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Synthesised cDNAs were kept at -80 °C for up to 10 days.

Quantitative real-time PCR was performed using TaqMan® 5'-nuclease gene expression assays (Applied Biosystems, Foster City, CA, USA) for human resistin gene (Hs00220767) and for IL-6 (Hs00985639) gene, while beta actin (Hs01060665) was used as the housekeeping gene. All samples were pooled and five different dilutions of the pool were used to obtain a standard curve for each target gene and for a housekeeping gene. 5 µL of cDNA was used as a template in a 15µL PCR reaction. The results were analysed in real-time mode using SDS Version 1.4.0.25 software. Data were expressed as a ratio between the target gene mRNA and the mRNA of the housekeeping gene: Normalised resistin mRNA= resistin mRNA/beta-actin mRNA; normalised IL-6 mRNA = IL-6 mRNA/ beta-actin mRNA.

Creatinine, glucose and lipid parameters (TC, HDL-C, LDL-C, Tg) were assayed from serum samples, by routine methods on a ILab 300+ analyser (Instrumentation Laboratory, Milan, Italy). hsCRP was measured from fresh serum samples, by a highly sensitive, latex particle-enhanced immunoassay (Roche Diagnostics, Mannheim, Germany). Intra-assay coefficient of variations (CV) for human serum were: CV (mean = 13.3 mg/L) =0.4%; CV (mean = 0.53 mg/L) = 1.6%, while inter-assay CVs were: CV (mean = 13.3 mg/L) = 2.1%; CV (mean = 0.53 mg/L) = 8.4%, as indicated by the manufacturer. Fasting insulin concentrations were measured from fresh serum samples by an electro-chemiluminescence immunoassay method, on a COBAS®INTEGRA 400 plus analyser – Roche Diagnostics: intra-assay CV (mean = 6.36 µU/L) = 1.9%; inter-assay CV (mean = 6.36 µU/L) = 2.6%, as indicated by the manufacturer. Insulin sensitivity was assessed using HOMA-IR [HOMA-IR = (fasting insulin µU/mL x fasting glucose mmol/L)/22.5].

Resistin and IL-6 protein concentrations were measured from thawed plasma samples using an enzyme linked immunosorbent assay in all subjects (ELISA, R&D systems, Wiesbaden, Germany) according to the manufacturer's protocol. In order to assess intra-assay precision for resistin, 20 replicates of pooled human plasma were run on one plate, CV (mean = 10.23 ng/mL) = 4.7%, while 10 replicates on three different plates were run in three days in order to assess inter-assay precision, CV (mean = 10.43 ng/mL) = 8.4%. In order to assess intra-assay precision for IL-6, 20 replicates of pooled human plasma were run on one plate, CV (mean = 3.83 pg/mL) = 2.6 %, while 10 replicates on three different plates were run in three days in order to assess inter-assay precision, CV (mean = 4.07 pg/mL) = 4.5%.

Statistical analysis

All data were analysed using IBM® SPSS® Statistics version 22 software. Distribution of data was tested using the Shapiro-Wilk test. All data are expressed as medians with interquartile ranges, while age is expressed as medians with min-max ranges. Groups were compared with the Kruskal-Wallis and the Mann-Whitney U tests with Bonferroni correction. Bonferroni adjustment was performed by multiplying the nominal P values with the number of tests performed. Spearman correlation analysis was used for data with skewed distributions (24). Chi square analysis and Fisher 2x3 exact tests were used for categorical variables. A P-value of less than 0.05 was considered statistically significant.

Results

Baseline data and laboratory characteristics of controls and patients are shown in Table 1. Bonferroni adjustment for all P values between groups was performed, and these values are further presented. All patients were significantly older than subjects in the CG. Serum creatinine was signifi-

Table 1. Clinical and laboratory characteristics of controls and patients

Variable	Control group	CAD negative patients	Stable angina pectoris	Acute coronary syndrome	P
Number of subjects	33	30	18	29	/
Gender (M/%)	15/45%	12/40%	10/56%	16/55%	0.602
Age (years)	45 (27-60)	62 (32-80)[a,***]	61 (52-74)[a,***]	58 (38-82)[a,***]	**<0.001**
BMI (kg/m^2)	24.7 (22.6-27.2)	25.8 (24.4-28.0)	26.2 (25.7-28.5)	26.7 (23.5-28.3)	0.208
Creatinine (μmol/L)	86 (78-94)	68 (59-84)[a,**]	60 (58-85)[a,*]	74 (63-88)[a,*]	**0.001**
Glucose (mmol/L)	5.1 (4.8-5.4)	5.2 (4.8-5.8)	5.1 (4.9-6.0)	5.5 (5.1-5.9)	0.102
TC (mmol/L)	4.80 (4.50-5.82)	4.72 (4.28-5.89)	4.61 (4.02-4.98)	4.53 (3.96-5.20)	0.256
LDL-C (mmol/L)	2.91 (2.52-3.54)[c,**]	3.07 (2.62-3.92)[c,**]	2.28 (2.17-2.60)	2.67 (2.35-3.50)	**0.003**
HDL-C (mmol/L)	1.54 (1.35-1.82)	1.12 (0.96-1.45)[a,***]	1.17 (1.07-1.38)[a,***]	1.05 (0.90-1.30)[a,***]	**<0.001**
Tg (mmol/L)	1.26 (0.99-1.64)	1.20 (1.02-1.52)[c,**,b*]	1.81 (1.39-2.26)[a,*]	1.66 (1.44-2.03)	**0.001**
hsCRP (mg/L)	1.16 (0.61-1.87)	2.00 (1.2-5.6)	3.09 (0.87-5.35)	4.55 (2.09-8.95)[a,**]	**<0.001**
Insulin (μU/L)	8.37 (6.59-10.89)[b,*]	9.10 (6.21-13.65)	7.06 (6.82-13.44)	11.03 (9.15-19.68)	**0.010**
HOMA-IR	1.89 (1.58-2.63)[b,**]	2.28 (1.32-2.99)[b,*]	2.74 (1.41-3.58)	3.43 (2.63-4.82)	**0.007**
IL-6 (pg/mL)	BDL	2.24 (0.79-3.29)	1.63 (1.00-5.58)	3.08 (1.21-8.82)	0.973
Normalised IL-6 mRNA	0.759 (0.462-1.138)	0.577 (0.297-0.860)	0.642 (0.445-0.736)	0.640 (0.407-0.938)	0.236
Resistin (ng/mL)	10.94 (7.79-13.01)[b,**]	11.58 (9.29-15.06)[b,*]	13.89 (10.46-15.06)	16.43 (11.76-23.41)	**<0.001**
Normalised resistin mRNA	0.725 (0.585-1.064)	0.695 (0.544-0.988)	0.774 (0.503-0.857)	0.684 (0.481-1.014)	0.988
Medication (with/without therapy)					
ACE-inhibitors (N/total)	/	20/10	16/2	18/11	0.143
Beta-blockers (N/total)	/	18/12	10/8	22/7	0.273
Diuretics (N/total)†	/	13/17	2/16	4/25	**0.015**
Statins (N/total)	/	8/22	6/12	15/14	0.133

Values are shown as medians with interquartile ranges (median, min-max range for age) and tested with Kruskal-Wallis test;
BMI - body mass index; TC - total cholesterol; LDL-C - low density lipoprotein cholesterol; HDL-C - high density lipoprotein cholesterol; Tg - triglycerides; hsCRP - high sensitivity C-reactive protein; HOMA-IR - homeostasis model assessment of insulin resistance; IL-6 - interleukin 6.
[a]significantly different compared to healthy subjects; [b]significantly different compared to ACS group; [c]significantly different compared to SAP group; *-P < 0.05; **-P < 0.01; ***-P < 0.001 after Bonferroni adjustment; BDL - below detection limit of the assay (< 0.7 pg/mL); †-P < 0.05 compared with Fisher 2x3 exact test.

cantly higher in the CG compared to CAD negative (P = 0.006), SAP (P = 0.012) and ACS groups (P = 0.024). LDL-C was lower in the SAP group compared to the CG (P = 0.006) and CAD negative patients (P = 0.006). HDL-C was higher in the CG compared to CAD negative (P < 0.001), SAP (P < 0.001) and ACS groups (P < 0.001). SAP and ACS groups had similar concentrations of Tg, both being higher than in the CAD negative group (P = 0.006 and P = 0.018, respectively), while SAP group had significantly higher Tg concentrations than the CG (P = 0.024). HsCRP was lower in the CG compared to ACS group (P = 0.001). Insulin was higher in the ACS group compared to the CG group (P = 0.012). HOMA-IR index was significantly higher in the ACS group compared to the CG (P = 0.006) and the CAD negative group (P = 0.048).

In contrast to that found in patients, IL-6 protein was present below the limit of detection (< 0.7 pg/mL) in all control plasma samples. IL-6 mRNA did not show any significant difference between the groups (Table 1).

Plasma resistin was significantly higher in the ACS group compared to the CG and CAD negative group (P = 0.001, P = 0.018, respectively, Table 1). However, resistin mRNA expression did not show any significant difference between any of the groups investigated (Table 1).

In patients plasma resistin correlated positively with creatinine and resistin mRNA but negatively with TC and HDL-C (Table 2). There was no correlation with BMI, glucose, insulin or HOMA-IR index, IL-6 protein and hsCRP (Table 2). However, patients that were not taking statin therapy showed positive correlation of plasma resistin with hsCRP (Table 2).

In patients resistin mRNA correlated positively with plasma resistin protein [this correlation was also observed in the CG (P = 0.046, ρ = 0.360)] and plasma IL-6, while negative correlation was observed with HDL-C (Table 2).

When plasma resistin was categorized into tertiles based on cut-off points of the entire patient distribution, a high frequency of ACS patients were observed in the third tertile. In the first and second tertiles CAD negative patients were abundant. The highest resistin tertile was accompanied by significantly lower TC and HDL-C compared to the patients in the lowest tertile (Table 3).

Discussion

For the first time PBMC resistin mRNA expression together with plasma resistin protein was investigated in patients with CAD symptoms. We demonstrated that plasma resistin was significantly elevated in the ACS group, compared to CG and CAD negative group. In contrast, resistin mRNA did not show any difference between the investigated groups. In patients plasma resistin was positively associated with creatinine and resistin mRNA but negatively with TC and HDL-C. Resistin mRNA showed positive association with IL-6 protein but negative correlation with HDL-C. The highest plasma resistin tertile included a high frequency of

Table 2. Correlations of plasma resistin and normalized resistin mRNA with other parameters in the patients group.

Parameter	Resistin protein		Normalised resistin mRNA	
	ρ	P	ρ	P
Age	0.214	0.062	0.026	0.827
BMI	-0.202	0.082	-0.098	0.410
Creatinine	0.353	**0.002**	0.166	0.156
Glucose	-0.050	0.678	-0.086	0.482
TC	-0.373	**0.001**	-0.111	0.356
HDL-C	-0.404	**<0.001**	-0.257	**0.032**
LDL-C	-0.231	0.051	-0.093	0.442
Tg	0.048	0.684	-0.032	0.791
hsCRP	0.124	0.288*	0.164	0.165
Insulin	0.014	0.911	0.052	0.693
HOMA-IR	-0.118	0.359	-0.086	0.512
IL-6	0.206	0.197	0.297	0.048
Normalised IL-6 mRNA	-0.056	0.636	0.026	0.823
Resistin	/	/	0.305	**0.008**
Normalised resistin mRNA	0.305	**0.008**	/	/

BMI - body mass index; TC - total cholesterol; LDL-C - low density lipoprotein cholesterol; HDL-C - high density lipoprotein cholesterol; Tg - triglycerides; hsCRP - high sensitivity C-reactive protein; HOMA-IR - homeostasis model assessment of insulin resistance; IL-6 - interleukin 6.

*Significant when patients on statin therapy were excluded: P = 0.047, ρ = 0.295

Table 3. Anthropometric, laboratory and gene expression data according to plasma resistin concentration tertiles in patients.

Parameter	Resistin tertiles			
	I tertile (6.17-10.90 ng/mL) N = 26	II tertile (10.91-16.16 ng/mL) N = 25	III tertile (16.17-38.66 ng/mL) N = 26	P
Patient subgroups § (SAP+CAD-/ACS)	19/7	19/6	9/17	**0.003**
Age (years)	59 (37-80)	60 (32-75)	66 (38-82)	0.128
BMI (kg/m^2)	26.7 (25.1-28.7)	25.8 (24.5-28.1)	26.6 (23.5-27.5)	0.550
Creatinine (μmol/L)	60 (54-78)	78 (60-87)	69 (64-97)	0.052
Glucose (mmol/L)	5.6 (4.9-6.0)	5.1 (4.7-5.5)	5.5 (5.1-5.9)	0.266
TC (mmol/L)	5.01 (4.53-5.97)	4.66 (4.14-5.73)	4.28 (3.68-4.81)*	**0.033**
HDL-C (mmol/L)	1.32 (1.08-1.45)	1.15 (0.90-1.47)	0.97 (0.87-1.20)**	**0.012**
LDL-C (mmol/L)	2.96 (2.44-4.07)	2.95 (2.40-3.70)	2.64 (2.21-3.01)	0.243
Tg (mmol/L)	1.39 (1.22-2.31)	1.46 (1.04-1.76)	1.64 (1.20-1.96)	0.501
hsCRP (mg/L)	2.40 (1.56-5.35)	2.37 (0.82-6.40)	5.31 (2.05-9.75)	0.222
Insulin (μU/L)	9.20 (7.04-11.06)	13.44 (6.82-16.57)	10.71 (6.05-16.50)	0.386
HOMA-IR	2.51 (2.20-3.20)	2.98 (1.52-3.73)	2.65 (1.33-3.96)	0.826
IL-6 (pg/mL)	1,83 (1,42-4,75)	2,46 (1,16-3,81)	7,25 (1,36-9,59)	0.288
Normalised IL-6 mRNA	0.642 (0.408-0.792)	0.538 (0.323-0.844)	0.543 (0.330-0.986)	0.642
Normalised resistin mRNA	0.648 (0.481-0.786)	0.731 (0.578-0.949)	0.834 (0.534-1.351)	**0.047**

Values are shown as medians with interquartile ranges (median, min-max range for age) and tested with Kruskal-Wallis test.
BMI - body mass index; TC - total cholesterol; LDL-C - low density lipoprotein cholesterol; HDL-C - high density lipoprotein cholesterol; Tg - triglycerides; hsCRP - high sensitivity C-reactive protein; HOMA-IR - homeostasis model assessment of insulin resistance; IL-6 - interleukin 6; CAD - coronary artery disease negative patients; SAP - stabile angina pectoris patients; ACS - acute coronary syndrome patients.
*significantly different compared to the I tertile: P = 0.027 after Bonferroni adjustment.
**significantly different compared to the I tertile: P = 0.006 after Bonferroni adjustment.
§ -values were analysed by Chi-square test.

ACS patients, but lower values of TC and HDL-C compared to patients in the lowest tertile.

Several *in vitro* experiments have indicated that resistin plays an important role in endothelial dysfunction, considered as one of the earliest stages of atherosclerosis development (6). Qiao and co-workers found that resistin plasma concentrations were higher in AMI, UAP and SAP patients compared to healthy subjects, even without adjustment for GFR or age (16). Lubos and colleagues also observed higher resistin plasma concentrations in ACS patients and indicated a potential use of resistin as a diagnostic marker. However, plasma resistin concentrations were not associated with future cardiovascular outcome (12,17). Herein we have shown that plasma resistin is strongly associated with serum creatinine, which is in agreement with the findings of other authors (14) and suggests that its metabolism is strongly dependent of kidney function.

Our study has revealed a positive association between PBMC resistin mRNA expression and circulating resistin in both the CG and CAD patients. Mezaghi and colleagues found a positive association between plasma resistin and PBMC resistin mRNA in type 2 diabetes patients (18). Tsiotra and co-workers reported the same observation between resistin mRNA expression from human peripheral monocyte enriched mononuclear cells and circulating resistin in type 2 diabetic women (25). However, PBMC resistin mRNA expression was similar between the examined study groups.

While the contribution by PBMCs to the resistin plasma concentrations is not to be underestimated, other factors beside kidney function can contribute to its final plasma concentrations. Resistin is secreted from macrophages, in atheroma, locally and abundantly where it exerts its effects within atherosclerotic lesions in a paracrine manner (6). Furthermore, it is commonly believed that activation of inflammatory cells in culprit stenosis is the cause of coronary instability in all patients (2). Additionally, some papers reported resistin's possible contribution to atherothrombosis by promoting tissue factor expression, thus representing an effector molecule able to induce a pro-thrombotic phenotype in cells present in the vessel wall (6). In such a way resistin could contribute to complex intravascular inflammatory and pro-thrombotic responses which are the major components leading to dynamic instability of a coronary atherosclerotic plaque, regarded as the foundation for the development of the clinical syndromes including UAP and AMI (2). This could explain why the highest resistin plasma concentrations, without PBMC resistin gene expression differences were observed in ACS patients: During plaque destabilisation, which takes its course in UAP and AMI, resistin protein could be additionally released from the plaque itself (6).

Other studies have examined connections between plasma resistin and HDL-C concentrations (12,13). We have shown for the first time that not only plasma resistin but also its PBMC mRNA are inversely related to HDL-C. Furthermore, patients with the highest plasma resistin, possess a more pro-atherogenic lipid status: HDL-C concentrations were the lowest in that group. In line with previously reported results (13), plasma resistin was negatively associated with TC and TC concentrations were the lowest in patients with the highest plasma resistin, which could be a consequence of the negative association observed with HDL-C concentrations. Sato and co-workers reported that mouse resistin protein can attenuate ApoA1 mRNA expression in the liver, which could be the cause of lower HDL-C concentrations seen in mice treated with resistin adenovirus (26). However, one should bear in mind the discrepancies that exist between human and mouse resistin proteins (3-5). The exact relationship between resistin, both plasma protein and PBMC mRNA expression, and HDL-C in humans remains to be clarified.

In other reports resistin has been shown to be positively associated with markers of inflammation, such as hsCRP (12,16,17) and IL-6 protein (27). We observed significant positive correlation between resistin and hsCRP, but only when excluding subjects that were taking statin therapy. Namely, statins are known to exert pleiotropic anti-inflammatory effects via HMG-CoA reductase-independent pathways. In particular simvastatin inhibits hsCRP-induced resistin expression in cultured human PBMCs via the mevalonate-GGPP pathway (28). This could explain why only patients that were not on lipid lowering therapy retained this correlation. However, we were not able to show that resistin was positively correlated with plasma IL-6. On the other hand, PBMC resistin mRNA expression positively correlated with plasma IL-6. This could be due to the fact that not only can resistin stimulate IL-6 expression, but IL-6 can stimulate resistin expression in PBMCs (7,8). Anderson and colleagues observed an increase in both IL-6 protein and PBMC IL-6 mRNA expression in AMI patients (29). We failed to confirm this, most likely due to different study design.

Some limitations within this study should be mentioned. First, we had small study group sizes. Our findings should be confirmed using larger study groups. Second, patients were older than the healthy subjects. Third, lipid parameters were measured over a rather short time after acute coronary events. Finally, all patients received some form of therapy (lipid lowering or anti-hypertensive medications), which could have influenced our results (6,30).

Conclusion

In this study we have observed significant increase of plasma resistin in patients with ACS compared to CG and CAD negative patients. Despite no change in PBMC resistin mRNA in different disease conditions a positive association between resistin mRNA and resistin plasma protein was evident.

Furthermore, a positive association between plasma resistin and serum creatinine concentrations was demonstrated. Both plasma resistin and PBMC resistin mRNA were negatively associated with plasma HDL-C.

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