



# Resistin level in coronary artery disease and heart failure: the central role of kidney function

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**Objectives** The aim of this study was to evaluate resistin levels in patients with coronary artery disease (CAD) with or without chronic heart failure, in order to define its independent predictor.

**Methods** One hundred and seven outpatients with CAD were enrolled in the study and divided into three groups: CAD without left-ventricular systolic dysfunction (group 1); CAD with left-ventricular dysfunction without heart failure symptoms (group 2); CAD with overt heart failure (group 3). Plasma resistin was determined by ELISA.

**Results** Resistin progressively increased from group 1 ( $10.7 \pm 5.0$  ng/ml) to groups 2 ( $11.8 \pm 5.8$  ng/ml) and 3 ( $17.0 \pm 6.8$  ng/ml), with the difference reaching statistical significance in group 3 versus groups 1 and 2 ( $P = 0.001$ ). A multivariable model of analysis demonstrated that the best predictor of plasma resistin level was the estimated glomerular filtration rate ( $P < 0.001$ ), indicating that reduction of kidney function was the main cause of the adipokine increase observed in patients with CAD and overt heart failure.

## Introduction

Adipose tissue is an active, endocrine organ that secretes numerous bioactive peptides, called adipokines. Adipokines affect several metabolic pathways<sup>1</sup> and are involved in glycometabolic homeostasis,<sup>2</sup> inflammation,<sup>3</sup> obesity,<sup>4</sup> atherosclerosis and ischemic heart disease.<sup>5</sup>

Resistin, which is produced in humans by adipocytes and monocytes,<sup>6</sup> is an esameric adipokine that circulates as high and low-molecular-weight isoforms, with different biological activities.<sup>4</sup> Resistin supports the atherosclerotic process through the production of pro-inflammatory cytokines,<sup>7</sup> such as interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1, mediated by the overexpression of nuclear factor (NF)- $\kappa$ B.<sup>8</sup> Resistin also promotes the expression of endothelin-1, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1 and several metalloproteinases, leading to the onset and progression of endothelial dysfunction.<sup>9</sup> In addition, resistin stimulates migration of macrophages and smooth muscle cells, from arterial media to the intima, with the consequent formation of foam cells.<sup>10</sup> Murine cardiomyocytes, incubated with

**Conclusions** Our data confirm the rise of resistin plasma levels previously described in patients affected by chronic heart failure; however, in our study, this relationship seemed to be mediated mainly by the level of kidney function, and only partially by the severity of ventricular dysfunction.

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**Keywords:** adipokine, chronic heart failure, coronary artery disease, kidney function, resistin

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resistin, show a tumor necrosis factor (TNF)- $\alpha$ -mediated glycometabolic dysregulation, resulting in cell damage, as demonstrated by the elevation of creatine phosphokinase (CPK) and troponin I.<sup>11</sup>

Clinical studies also reported elevated resistin concentrations in patients with chronic coronary artery disease (CAD)<sup>12</sup> and acute coronary syndromes, suggesting a role for this adipokine as a marker of myocardial ischemia *in vivo*.<sup>13</sup> Increased resistin levels predicted the incidence of myocardial infarction<sup>14</sup> and were associated with a higher risk of re-stenosis after coronary angioplasty.<sup>15</sup> Furthermore, resistin was inversely correlated with left-ventricular ejection fraction (LVEF), and directly correlated with measures of myocardial damage, during myocardial infarction;<sup>13</sup> in this setting, elevated plasma levels of the adipokine predicted mortality.<sup>16</sup>

Lastly, recent data from the Framingham Offspring Study demonstrated that levels of resistin above 15 ng/ml were associated with an increased risk of developing heart failure.<sup>17</sup>

Although experimental studies and longitudinal surveys suggest that elevated resistin could contribute to myocardial damage in patients with ischemic heart disease, thus contributing to the pathogenesis of heart failure, the possibility that reduced cardiac function is also a cause, and not only a consequence, of the increased circulating resistin cannot be dismissed.

The aim of this study was to evaluate resistin in patients with CAD, with normal systolic function, with asymptomatic left-ventricular dysfunction or with overt chronic heart failure, and to identify its independent predictor, in order to shed more light on the complex relationship that exists between ischemic heart disease, cardiac function and circulating levels of this adipokine.

## Methods

### Study participants

A consecutive series of Caucasian outpatients referred for CAD to the Geriatric Cardiology and Medicine Unit of Careggi Academic Hospital (Florence, Italy), between 1 January 2006 and 31 December 2008, were enrolled, provided that they met the following inclusion criteria: written informed consent, CAD documented by at least one 75% stenosis of a major epicardial branch at coronary angiography, no hospitalization for worsening heart failure during the past 2 months. The study was approved by an institutional review committee.

### Clinical and instrumental data

A thorough clinical history, including all medications used by the patient and symptoms needed to define New York Heart Association (NYHA) class, was collected. Patients underwent a physical examination with measurement of height and weight to calculate body mass index (BMI), 12-lead electrocardiogram (ECG), and 6-min walking test.<sup>18</sup> A standard echocardiographic study was carried out following the American Society of Echocardiography recommendations,<sup>19</sup> using a 4-chamber apical view to calculate LVEF, according to the Simpson's formula.<sup>19</sup> Heart failure was considered to be overt in the presence of positive European Society of Cardiology (ESC) criteria,<sup>20</sup> coupled with a Boston score of at least 5.<sup>21</sup> Combining LVEF with ESC and Boston criteria, patients were classified as group 1 (LVEF  $\geq$ 55%, no overt heart failure;  $n=46$ ), 2 (LVEF  $<$ 40%, no overt heart failure;  $n=24$ ), and 3 (LVEF  $<$ 40%, overt heart failure;  $n=37$ ). Patients with LVEF in the 40–54% range were excluded from the study in order to select patients with markedly different left-ventricular systolic function.

The level of noncardiac comorbidity was measured with the Greenfield's Index of Disease Severity (IDS score),<sup>22</sup> whereas the presence of depressive symptoms was evaluated with the 30-item form of the Geriatric Depression Scale (GDS).<sup>23</sup> Diabetes was diagnosed according to American Diabetes Association (ADA) criteria.<sup>24</sup>

### Biochemical data

Venous blood samples were drawn in the fasting state to determine glycemia, hemoglobin, glycosylated hemoglobin (HbA1c, determined with the high liquid pressure chromatography method; Menarini Diagnostics, Florence, Italy; upper normal limit 5.9%), erythrocyte sedimentation rate (ESR), blood urea nitrogen (BUN), creatinine, uric acid, fibrinogen and lymphocyte count. Glomerular filtration rate (GFR) was estimated according to the Modification of Diet in Renal Disease (MDRD) Study method (eGFR).<sup>25</sup> Circulating plasma resistin was determined, in duplicate, by ELISA (Linco Research, Missouri, USA); N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured with a chemiluminescent immunoassay kit (Roche Diagnostic Laboratory, Indianapolis, Indiana, USA) on an Elecsys 2010 analyzer.

### Statistical analysis

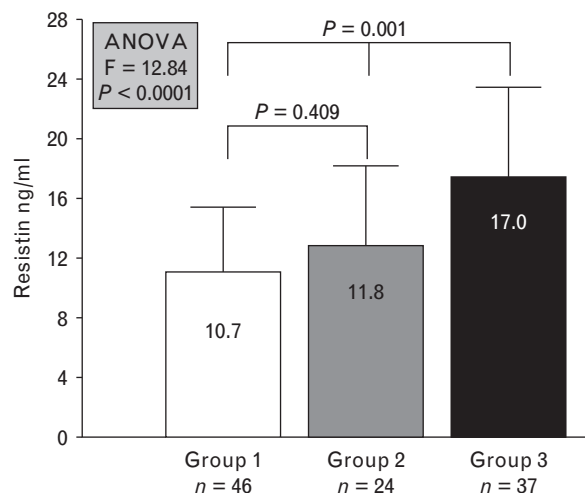
Data were analyzed using SPSS software (version 17; SPSS Inc., Chicago, Illinois, USA) and are presented as mean  $\pm$  SD or median (quartiles), depending on their normal or non-normal distribution, for continuous variables, and as number (%) for categorical ones. Categorical and continuous variables were compared across the three groups (1, 2 and 3) using chi-square test and analysis of variance (ANOVA), respectively. The association of resistin levels with demographic, clinical, pharmacological, echocardiographic and biohumoral variables, after adjusting for age, sex and BMI, was verified using a stepwise multiple linear regression model. For each aspect (demographic, clinical, pharmacological, echocardiographic and biohumoral), variables that showed the best association with resistin were entered into a multivariable, stepwise regression model, with re-coding categorical variables as 0/1 dummy variables, to sort those with the highest significant independent correlation. In case of more than one statistically significant association within a predefined aspect, only one variable was included in the regression model, to avoid co-linearity; this sorted variable had the highest  $\beta$  coefficient/SE ratio (Wald test). Finally, differences in plasma resistin levels across the three groups were adjusted in the ANOVA model using as covariate the best independent predictor of plasma resistin levels. A *P* value less than 0.05 was considered statistically significant.

## Results

### Characteristics of the study population

One hundred and seven patients, aged  $67.7 \pm 10.3$  years (range 38–90), were enrolled in the study; 13 were women (11.8%); mean value of BMI was  $26.7 \pm 4.0$  kg/m<sup>2</sup>. Cardiovascular comorbidity was distributed as follows: 74% of patients had previous myocardial infarction; 46% underwent myocardial revascularization (cardiac bypass surgery or coronary angioplasty); 19% had persistent/permanent

Fig. 1



Plasma levels of resistin in the three heart failure groups.

atrial fibrillation. Hypertension was present in 66%, diabetes in 42% and hypercholesterolemia in 68% of patients.

The mean distance walked in 6 min was  $454.9 \pm 164.0$  m; mean LVEF was  $44.1 \pm 15.3\%$ , 20.9% of patients were classified as NYHA III–IV. Among biohumoral variables, mean fasting glucose was  $120.4 \pm 47.4$  mg/dl, BUN  $54.8 \pm 30.0$  mg/dl, eGFR  $70.6 \pm 27.4$  ml/min, total cholesterol  $176.0 \pm 41.4$  mg/dl, uric acid  $5.9 \pm 1.8$  mg/dl and hemoglobin level  $12.04 \pm 4.74$  mg/dl. Mean NT-proBNP concentration was  $2154.3 \pm 4580.9$  ng/l.

Mean plasma resistin concentration was  $13.4 \pm 6.5$  ng/ml, with a significantly higher level in group 3 patients in comparison with groups 1 and 2 (Fig. 1). According to evidence-based guidelines, the majority of patients were treated with angiotensin-converting enzyme (ACE)-inhibitors/angiotensin receptor blockers (ARBs) (87%), beta-blockers (80%) and antiplatelet agents (83%).

#### Differences across the three groups

As reported in Table 1, patients in group 3 were significantly older, had lower BMI, higher comorbidity, less frequently underwent percutaneous coronary angioplasty, and presented more frequently depressive symptoms and a history of atrial fibrillation. Patients in groups 2 and 3 had a higher rate of previous myocardial infarction. As expected, indicators of symptomatic heart failure, such as high NYHA class, signs and symptoms of pulmonary and systemic fluid retention, were more commonly detected in group 3 patients. Patients in groups 2 and 3 had echocardiographic parameters indicating enlargement of cardiac chambers. Moving from group 1 to 3 we registered a progressive reduction of E wave deceleration time (Tdec) and an increase in E/A

ratio, suggestive of worsening left-ventricular diastolic function. Patients in group 3 had a significantly higher tricuspid pressure gradient, an indirect indicator of elevated pulmonary artery pressure. Among the biohumoral variables, a significant, progressive reduction in eGFR was registered from group 1 to group 3, with a parallel progressive increase of BUN. All three biohumoral inflammatory markers (uric acid, fibrinogen and ESR) showed a gradual increase across the three groups (Table 1). Lastly, according to clinical severity, NT-proBNP progressively increased from group 1 to group 3.

Group 3 patients were treated more frequently with digoxin, diuretics, nitrates and warfarin, and less frequently with calcium antagonists, statins and antiplatelet agents. No significant differences were detected in the use of ACE/ARB or beta-blockers among the three groups.

As reported in Fig. 1, plasma resistin levels showed a progressive elevation from group 1 to 3, with the difference reaching statistical significance in group 3, compared with groups 1 and 2 ( $P=0.001$  and  $P=0.001$ , respectively).

#### Predictors of plasma resistin level

As reported in Table 2, in the anamnestic-demographic aspect, a statistically significant association was found between comorbidity and higher resistin; in the clinical functional aspect, increasing resistin level was associated with decreasing of distance walked in 6 min and higher NYHA class; in the echocardiographic aspect the E/A ratio was the only independent predictor, whereas in the biohumoral one eGFR and ESR were both able to predict resistin levels. Finally, in the pharmacological aspect, the only predictor was the use of digoxin. When the best predictors of each aspect were pooled in the full model of analysis (Table 3), the strongest indicator of plasma resistin level was eGFR, suggesting that the reduction of kidney function was the main cause of adipokine increase ( $P<0.001$ ). The highly significant, inverse relation between eGFR and resistin levels is reported in Fig. 2. In the adjusted ANOVA model, including eGFR as covariate, the difference in plasma resistin levels across the three groups still remained statistically significant ( $P=0.03$ ) (Table 4).

#### Discussion

In the pathogenesis of atherosclerosis the role of inflammation has been clearly established,<sup>26</sup> resistin may represent the link between inflammatory mediators and endothelial promoters of atherogenesis.<sup>3</sup> Indeed, expression of resistin is up-regulated during monocyte-macrophage differentiation,<sup>27</sup> and pro-inflammatory mediators, such as TNF- $\alpha$ , stimulate the expression of resistin in peripheral blood mononuclear cells.<sup>28</sup> In turn, resistin promotes endothelial cell activation through the production of adhesion molecules and monocyte

Table 1 Characteristics of the study population

	Group 1 (n = 46)	Group 2 (n = 24)	Group 3 (n = 37)	P value
Anamnestic and demographic aspect				
Age, years	67.1 ± 8.1	61.5 ± 11.3	72.4 ± 9.9	0.001
BMI, kg/m <sup>2</sup>	27.9 ± 3.2	26.6 ± 5.4	25.3 ± 3.2	0.012
Female sex	13.0	8.3	3.5	0.788
Previous myocardial infarction	60.8	87.5	86.4	0.008
Previous angina	52.2	41.7	35.1	0.287
Previous CABG	28.3	29.2	27.0	0.993
Previous PCI	76.1	87.5	59.5	0.053
Smokers	54.3	54.2	45.9	0.918
Hypertension	71.7	54.2	73.0	0.242
Hypercholesterolemia	76.1	75.0	59.5	0.217
Atrial fibrillation	17.4	0.0	35.1	0.003
Diabetes	52.2	29.2	37.8	0.112
COPD	4.3	8.3	21.6	0.041
Comorbidity level-IDS score	2.5 ± 1.8	2.3 ± 2.4	4.2 ± 1.8	<0.001
Depressive symptoms-GDS score	6.0 ± 4.8	6.6 ± 4.8	10.1 ± 5.6	0.002
Clinical and functional aspect				
Distance at 6-WT, m	499.0 ± 134.8	517.7 ± 156.1	340.0 ± 154.3	0.001
NYHA class	1.1 ± 0.2	1.1 ± 0.3	2.7 ± 0.7	<0.001
Jugular vein distension	4.3	0.0	29.0	0.001
Third heart sound	0.0	0.0	18.9	0.001
Pulmonary rates	8.7	0.0	43.2	0.001
Leg edema	6.5	8.3	35.1	0.001
Echocardiographic aspect				
Left atrial diameter, mm	39.7 ± 5.2	42.4 ± 5.7	46.9 ± 7.4	0.001
End systolic diameter, mm	52.7 ± 4.3	63.9 ± 14.3	69.5 ± 7.0	0.001
End diastolic diameter, mm	35.4 ± 5.8	50.9 ± 7.3	58.2 ± 7.4	0.001
IVRT, ms	87.9 ± 23.8	102.1 ± 25.1	91.9 ± 17.2	0.073
E/A ratio	1.0 ± 0.3	1.1 ± 0.8	2.1 ± 2.0	0.001
Ejection fraction	59.8 ± 6.4	34.4 ± 4.9	29.1 ± 4.5	<0.001
Tdec, ms	215.3 ± 57.1	202.4 ± 52.5	183.8 ± 81.6	0.135
Myocardial mass g/m <sup>2</sup>	110.5 ± 22.1	142.2 ± 37.8	148.0 ± 30.7	0.001
Trans-tricuspidal gradient, mmHg	8.5 ± 13.6	7.1 ± 15.2	20.9 ± 21.9	0.003
Biohumoral aspect				
Fasting blood glucose, mg/dl	121.2 ± 47.2	117.7 ± 32.5	121.3 ± 56.7	0.950
eGFR, ml/min	81.9 ± 29.8	73.8 ± 19.9	55.7 ± 22.1	0.001
Hemoglobin, g/dl	13.9 ± 1.3	13.7 ± 1.7	13.1 ± 1.6	0.075
HbA1c, %	6.8 ± 1.5	6.3 ± 1.0	6.7 ± 1.3	0.467
ESR, mm/h	27.6 ± 17.8	33.2 ± 25.0	51.4 ± 30.1	0.001
Fibrinogen, mg/dl	410.9 ± 94.1	432.3 ± 118.3	478.8 ± 98.8	0.015
Uric acid, mg/dl	5.3 ± 1.3	6.1 ± 1.7	6.5 ± 2.1	0.009
Lymphocyte, %	27.2 ± 8.3	28.1 ± 9.2	24.0 ± 12.2	0.256
Total cholesterol, mg/dl	174.1 ± 35.0	187.8 ± 45.7	170.6 ± 45.0	0.269
HDL, mg/dl	43.6 ± 10.8	44.8 ± 9.9	40.9 ± 11.6	0.360
BUN, mg/dl	45.9 ± 21.7	51.0 ± 22.6	68.2 ± 37.8	0.003
NT-proBNP, ng/l	582.3 ± 1022.1	2108.6 ± 3798.0	4367.2 ± 6803.5	0.002
Pharmacological aspect				
ACE-inhibitors/ARB	87.0	96.0	86.5	0.434
Beta-blockers	73.9	92.0	83.8	0.157
Diuretics	28.3	56.0	89.2	0.001
Antiplatelet agents	89.1	100.0	67.6	<0.001
Digoxin	8.7	16.0	45.9	<0.001
Warfarin	13.0	4.2	32.4	0.009
Antialdosterone agents	6.5	48.0	45.9	0.001
Calcium antagonists	43.5	8.0	10.8	0.001
Nitrates	26.1	20.0	48.6	0.029
Statins	80.4	80.0	57.8	0.035

Data are expressed as means ± SD for continuous and as % for categorical variables. 6-WT, 6-min walking test; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BUN, blood urea nitrogen; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; GDS, geriatric depression scale; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IDS, index of disease severity; IVRT, isovolumic relaxation time; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; Tdec, transmitral E-wave deceleration time.

chemoattractant protein (MCP)-1, promoting atherogenesis.<sup>9</sup> Resistin was detected in macrophages within atherosclerotic lesions,<sup>7</sup> and, recently, Xu *et al.*<sup>29</sup> demonstrated an involvement of the adipokine in macrophage CD36 expression and lipid accumulation.<sup>30</sup>

Taken together, these data suggest a possible pathophysiological role of resistin in cardiovascular disease

progression. Indeed, resistin appeared to mediate the myocardial damage induced by ischemia/reperfusion<sup>11</sup> and to alter cardiomyocyte contractility and relaxation velocities through the impairment of cytoplasmic calcium clearing.<sup>31</sup> In addition, the adipokine correlated with the risk of re-stenosis after coronary stenting in diabetic patients,<sup>15</sup> and predicted mortality after acute myocardial infarction.<sup>16</sup>

**Table 2 Relationships between resistin level and variables grouped into anamnestic-demographic, clinical and functional, echocardiographic, biohumoral and pharmacological aspects**

Variable	Model 1* Beta (SE)	P value	Model 2§ Beta (SE)	P value
Anamnestic and demographic aspect			<i>R</i> = 0.65, <i>P</i> < 0.001	
Previous myocardial infarction (yes vs. no)	6.15 (6.70)	0.361		
Previous PCI (yes vs. no)	-0.04 (1.45)	0.997		
Atrial fibrillation (yes vs. no)	1.08 (1.58)	0.497		
COPD (yes vs. no)	2.32 (2.00)	0.250		
Comorbidity level-IDS score	1.05 (0.30)	0.001	1.03 (0.31)	0.001
Depressive symptoms-GDS score	0.37 (0.12)	0.004	-	-
Clinical and functional aspect			<i>R</i> = 0.54, <i>P</i> < 0.001	
Distance at 6-WT, m	-0.01 (0.01)	<0.001	-0.01 (0.00)	0.029
NYHA class	2.92 (0.67)	<0.001	2.55 (0.74)	0.001
Jugular vein distension (yes vs. no)	2.06 (2.03)	0.202		
Third heart sound (yes vs. no)	4.97 (2.41)	0.043	-	-
Pulmonary rates (yes vs. no)	2.91 (1.71)	0.092		
Leg edema (yes vs. no)	0.46 (1.75)	0.791		
Echocardiographic aspect			<i>R</i> = 0.52, <i>P</i> < 0.001	
Left atrial diameter, mm	0.22 (0.09)	0.020	-	-
End systolic diameter, mm	0.12 (0.05)	0.015	-	-
End diastolic diameter, mm	0.13 (0.05)	0.007	-	-
E/A ratio	1.31 (0.52)	0.015	2.00 (0.55)	0.001
Ejection fraction	-0.14 (0.04)	0.001	-	-
Myocardial mass g/m <sup>2</sup>	0.02 (0.02)	0.153	-	-
Trans-tricuspidal gradient, mmHg	0.09 (0.04)	0.010	-	-
Biohumoral aspect			<i>R</i> = 0.70, <i>P</i> < 0.001	
Fasting blood glucose, mg/dl	-0.03 (0.01)	0.046	-	-
HbA1c, %	-0.83 (0.49)	0.098	-	-
eGFR	-0.14 (0.02)	<0.001	-0.14 (0.02)	<0.001
Hemoglobin	-1.08 (0.42)	0.011	-	-
ESR	0.09 (0.03)	0.001	0.05 (0.02)	0.049
Fibrinogen	0.02 (0.01)	0.004	-	-
Uric acid	0.65 (0.36)	0.074	-	-
Lymphocyte count	-0.13 (0.06)	0.041	-	-
BUN	0.09 (0.02)	<0.001	-	-
NT-proBNP	0.01 (0.01)	0.031	-	-
Pharmacological aspect			<i>R</i> = 0.42, <i>P</i> < 0.001	
Diuretics (yes vs. no)	2.66 (1.22)	0.032	-	-
Antiplatelet agents (yes vs. no)	-2.35 (1.69)	0.167	-	-
Digoxin (yes vs. no)	4.08 (1.44)	0.006	4.46 (1.41)	0.002
Antialdosterone agents (yes vs. no)	1.04 (1.37)	0.451	-	-
Calcium antagonists (yes vs. no)	-0.21 (1.47)	0.888	-	-
Nitrates (yes vs. no)	0.70 (1.33)	0.600	-	-
Warfarin (yes vs. no)	3.21 (1.55)	0.041	-	-
Statins (yes vs. no)	-2.17 (1.41)	0.126	-	-

Model 1: adjusted for age, sex, BMI; model 2: stepwise backward regression model. Abbreviations: 6-WT, 6-min walking test; BMI, body mass index; BUN, blood urea nitrogen; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; GDS, geriatric depression scale; HbA1c, glycated hemoglobin; IDS, index of disease severity; IVRT, isovolumic relaxation time; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; Tdec, transmitral E-wave deceleration time.

In our clinical setting, patients with CAD, without left-ventricular systolic dysfunction, showed levels of resistin ( $10.7 \pm 4.9$  ng/ml) significantly higher than what was previously reported in healthy or in diabetic and nondiabetic, obese patients,<sup>32</sup> even higher than other series of patients

**Table 3 Independent predictors of plasma resistin level**

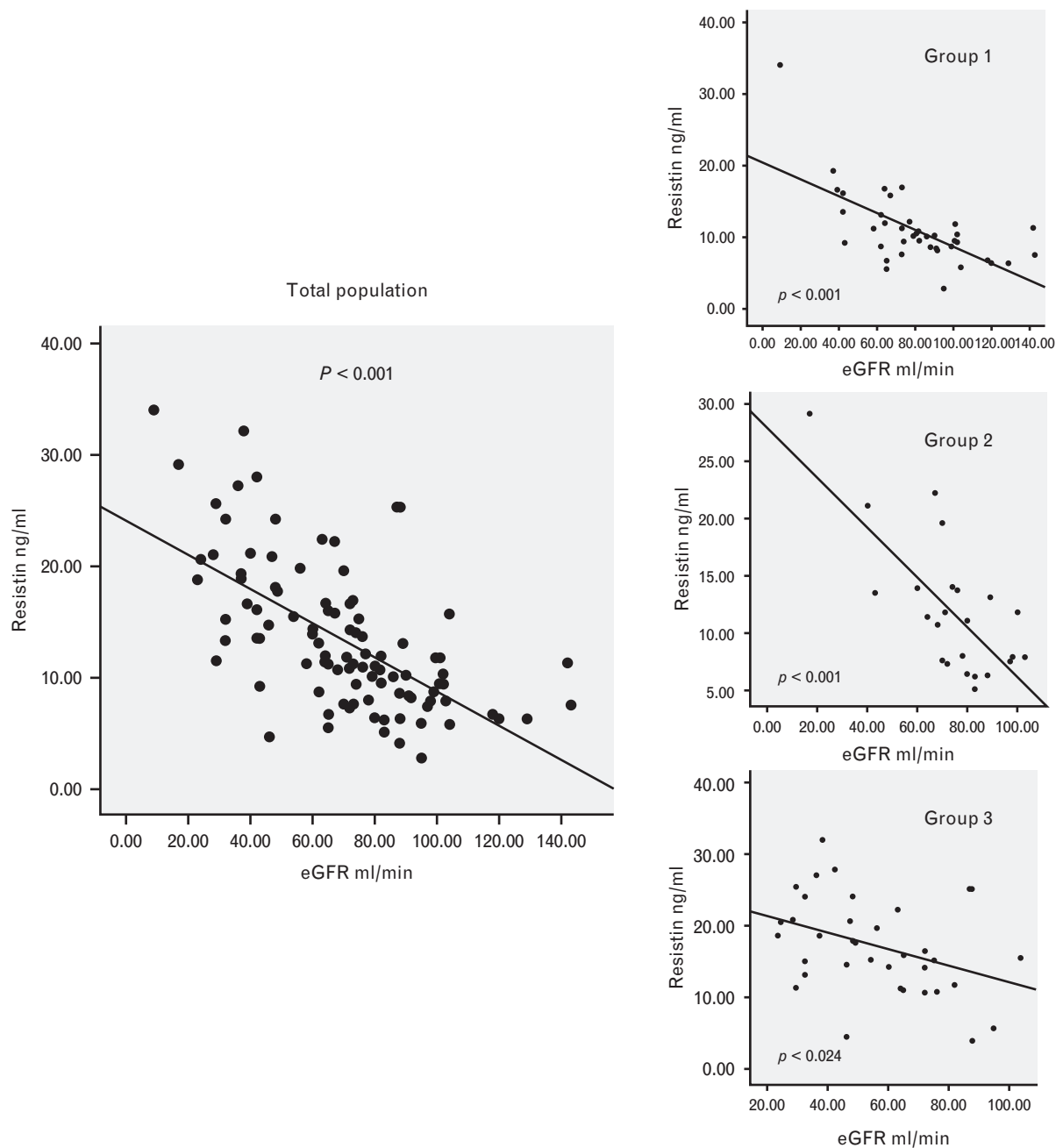
Multivariable model	<i>R</i> = 0.74 Beta (SE)	<i>P</i> < 0.001 <i>P</i> value
Age	0.05 (0.06)	0.409
Sex	1.02 (1.63)	0.535
BMI	-0.03 (0.15)	0.829
Comorbidity-IDS score	0.27 (0.30)	0.361
NYHA class	0.47 (0.84)	0.581
E/A ratio	0.19 (1.35)	0.911
Digoxin	2.35 (1.52)	0.127
eGFR	-0.14 (0.02)	<0.001

BMI, body mass index; eGFR, estimated glomerular filtration rate; IDS, index of disease severity; NYHA, New York Heart Association.

with CAD.<sup>33</sup> This finding could be explained by the fact that our study population was older, and included patients with chronic kidney disease that were excluded by other studies.<sup>33</sup> Moreover, in our patients, resistin level correlated strongly with IDS score, a measure of comorbidity, and with inflammatory markers (ESR and fibrinogen). A correlation with inflammation has been reported previously in patients with cardiovascular disease<sup>33</sup> and, more recently, in septic and nonseptic critically ill patients.<sup>34</sup>

We detected a progressive increase in mean plasma resistin levels from group 1 to group 3, although the difference became statistically significant only in group 3. In our patients with overt heart failure, higher levels of resistin correlated with indicators of functional capacity, such as NYHA class and distance walked in 6 min. In a cohort of hospitalized heart failure patients Takeishi

Fig. 2



Correlation between resistin and kidney function estimated by glomerular filtration rate (eGFR), in the total study population and in the three groups.

Table 4 Unadjusted and adjusted ANOVA models of the difference in plasma resistin levels among the three groups

Variable	Model 1		Model 2	
	F	P value	F	P value
Group classification	12.84	<0.0001	3.65	0.03
eGFR			40.10	<0.0001

Model 1: ANOVA unadjusted. Model 2: ANOVA adjusted for eGFR.

*et al.*<sup>35</sup> found a progressive increase in resistin levels according to NYHA class, from a mean value of 10 ng/ml in NYHA I, to a mean value of more than 20 ng/ml in NYHA IV. They also demonstrated that patients with plasma resistin levels over 14 ng/ml had a significantly higher risk of death.<sup>35</sup> Interestingly, in our series the mean value of resistin ( $17.0 \pm 6.8$  ng/ml) in group 3 patients, which were classified as NYHA III–IV, was

very similar to what was reported in the Takeishi *et al.* series.

Estimated GFR, a sensitive indicator of kidney function, was the main independent predictor of plasma resistin level. This is an interesting finding because it links the adipokine increase with the progressive reduction in renal perfusion that often accompanies chronic heart failure. The central role of eGFR in the rise of plasma resistin levels was clearly described by Ellington *et al.*<sup>36</sup> in patients with hypertension, in whom higher resistin is associated with lower eGFR. The association was independent from the presence of CAD risk factors, insulin resistance and inflammation.<sup>36</sup> Similar results were also reported by Axelsson *et al.*<sup>37</sup> in a post-hoc, cross-sectional study of 239 chronic kidney disease (CKD) patients. In this setting, serum levels of resistin were markedly elevated in CKD patients with mild to moderate (eGFR  $23.2 \pm 1.0$  ng/ml) and severe (eGFR  $39.9 \pm 1.3$  ng/ml) renal impairment, compared to controls (eGFR  $8.5 \pm 0.7$  ng/ml;  $P < 0.001$ ).

The strong association of resistin with eGFR may be explained by the ability of the adipokine, which is a low-molecular-weight plasma protein, to freely filter at the glomerulus; thus, a decrease in glomerular function will result in an elevation of plasma resistin concentration. However, using a mouse model of uremia, it has been demonstrated that the production of reactive oxygen species (ROS) that accompanies renal insufficiency, stimulated adipocytes to overexpress and release resistin.<sup>38</sup> Therefore, the link between kidney dysfunction and elevated plasma resistin concentrations may rely on more complex pathways than just glomerular filtration. Further longitudinal studies will be necessary to confirm the central role of kidney function in mediating resistin increase in patients with heart failure. Moreover, in our series, the interaction between severity of left-ventricular dysfunction and resistin remained significant even after adjusting for eGFR, indicating that the rise of adipokine concentration is not fully explained by the level of kidney function; the severity of heart failure *per se* or other cytokines/adipokines not tested in this study (i.e. TNF- $\alpha$ ) may contribute to the increase of resistin.

A cross-sectional study such as this cannot establish causal relationships; therefore, this investigation cannot provide definitive conclusions on the pathophysiological link between elevated resistin and myocardial dysfunction. However, there is evidence that resistin can acutely impair cardiac muscle glucose utilization and cardiomyocyte function.<sup>31,39</sup>

In addition, a further detrimental effect of resistin in heart failure patients can be postulated based on the results of Cohen *et al.*<sup>40</sup> demonstrating that in uremic and diabetic patients resistin was able to interfere with the chemotactic movement and the oxidative burst of polymorphonuclear leukocytes, impairing the immune

response. Therefore, resistin could contribute to the dysregulation of the immune system which is often detected in patients with advanced, chronic heart failure,<sup>41</sup> increasing the susceptibility to lethal infections, potentially affecting the prognosis of patients.<sup>42</sup>

Because in the present survey eGFR was the main predictor of resistin concentrations, it can be speculated that a vicious cycle could exist in patients with heart failure, with elevated resistin contributing to the deterioration of myocardial function, leading to an impairment of renal blood flow, which, in turn, induces an increase in resistin levels.

Some limitations of the present study need to be mentioned: first, women represent only 12% of the entire study population; second, the design of the study is cross-sectional, precluding the analysis of a cause-effect correlation between impaired kidney function and resistin; third, no data have been collected on different circulating resistin isoforms to evaluate their biological activity and their relationship with eGFR; and, fourth, the relation of resistin with other cytokines, such as TNF- $\alpha$  and IL-6, was not tested.

In conclusion, our results confirm the elevated resistin levels in CAD patients with overt heart failure compared to those with normal systolic function or with systolic dysfunction without any sign of heart failure. This relationship seems to be mainly, but not completely, mediated by kidney function and, specifically, by the level of GFR. If longitudinal studies confirm this finding, resistin should be considered an important player in the metabolic mechanisms that link atherosclerosis, the onset and progression of renal insufficiency and the severity of heart failure in coronary artery disease patients.

## References

- Ronti T, Lupatelli G, Mannarino E. The endocrine function of adipose tissue: an update. *Clin Endocrinol* 2006; **64**:355–365.
- Rajala MW, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule- $\beta$  selectively impair insulin action on glucose production. *J Clin Invest* 2003; **111**:225–230.
- Filkova M, Haluzik M, Gay S, Senolt L. The role of resistin as regulator of inflammation: implications for various human pathologies. *Clin Immunol* 2009; **133**:157–170.
- Steppan CM, Bailey ST, Bhat S, *et al.* The hormone resistin links obesity to diabetes. *Nature* 2001; **409**:307–312.
- Reilly MP, Lehrke M, Wolfe ML, *et al.* Resistin in an inflammatory marker of atherosclerosis in humans. *Circulation* 2005; **111**:932–939.
- Savage DB, Sewter CP, Klenk ES, *et al.* Resistin /FIZZ3 expression in relation to obesity and peroxisome proliferator-activated receptor- $\gamma$  action in humans. *Diabetes* 2001; **50**:2199–2202.
- Jung HS, Park KH, Cho YM, *et al.* Resistin is secreted from macrophages in atherosclerosis and promotes atherosclerosis. *Cardiovasc Res* 2006; **69**:76–85.
- Kralisch S, Sommer G, Stangl V, *et al.* Secretory products from human adipocytes impair endothelial function via nuclear factor kappaB. *Atherosclerosis* 2008; **196**:523–531.
- Verma S, Li SH, Wang CH, *et al.* Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation* 2003; **108**:736–740.
- Calabro P, Samudio I, Willerson JT, Yeh HT. Resistin promotes smooth muscle cell proliferation through activation of extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3-kinase pathways. *Circulation* 2004; **110**:3335–3340.

- 11 Rothwell SE, Richards AM, Pemberton CJ. Resistin worsens cardiac ischaemia-reperfusion injury. *Biochem Biophys Res Commun* 2006; **349**:400–407.
- 12 Pischon T, Bamberger CM, Kratzsch J, et al. Association of plasma resistin levels with coronary heart disease in women. *Obes Res* 2005; **13**:1764–1771.
- 13 Chu S, Ding W, Li K, Tang C. Plasma resistin associated with myocardium injury in patients with acute coronary syndrome. *Circ J* 2008; **72**:1249–1253.
- 14 Weikert C, Westphal S, Berger K, et al. Plasma resistin levels and risk of myocardial infarction and ischemic stroke. *J Clin Endocrinol Metab* 2008; **93**:2647–2653.
- 15 On YK, Park HK, Hyon MS, Jeon ES. Serum resistin as a biological marker for coronary artery disease and restenosis in type 2 diabetic patients. *Circ J* 2007; **71**:868–873.
- 16 Lee SH, Ha JW, Kim JS, et al. Plasma adiponectin and resistin levels as predictors of mortality in patients with acute myocardial infarction: data from infarction prognosis study registry. *Coron Artery Dis* 2009; **20**:33–39.
- 17 Frankel DS, Vasan RS, D'Agostino RB Sr, et al. Resistin, adiponectin, and risk of heart failure the Framingham offspring study. *J Am Coll Cardiol* 2009; **53**:754–762.
- 18 Guyatt GH, Sullivan MJ, Thompson PJ, et al. The 6-min walk: a new measure of exercise capacity in patients with chronic heart failure. *Can Med Assoc J* 1985; **132**:919–923.
- 19 Lang RM, Bierig M, Devereux RB, et al. Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005; **18**:1440–1463.
- 20 Swedberg K, Cleland J, Dargie H, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary [update 2005]: The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. *Eur Heart J* 2005; **26**:1115–1140.
- 21 Di Bari M, Pozzi C, Cavallini MC, et al. The diagnosis of heart failure in the community. Comparative validation of four sets of criteria in unselected older adults: the ICARE Dicomano study. *J Am Coll Cardiol* 2004; **44**:1601–1608.
- 22 Greenfield S, Blanco DM, Elashoff RM, Ganz PA. Development and testing of a new index of comorbidity. *Clin Res* 1987; **A35**:346.
- 23 Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. *J Psychiatr Res* 1982; **17**:37–49.
- 24 Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; **20**:1183–1197.
- 25 Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**:461–470.
- 26 Libby P, Ridker PM, Hansson GK. Leduq Transatlantic Network on atherothrombosis inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* 2009; **54**:2129–2138.
- 27 Patel L, Buckels AC, Kinghorn IJ, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003; **300**:472–476.
- 28 Bokarewa M, Nagaev I, Dahlberg L, et al. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* 2005; **174**:5789–5795.
- 29 Xu W, Yu L, Zhou W, Lou M. Resistin increases lipid accumulation and CD36 expression in human macrophages. *Biochem Biophys Res Commun* 2006; **351**:376–382.
- 30 Collot-Teixeira S, Martin J, McDermott-Roe C, et al. CD36 and macrophages in atherosclerosis. *Cardiovasc Res* 2007; **75**:468–477.
- 31 Kim M, Oh JK, Sakata S, et al. Role of resistin in cardiac contractility and hypertrophy. *J Mol Cell Cardiol* 2008; **45**:270–280.
- 32 Heilbronn LK, Rood J, Janderova L, et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 2004; **89**:1844–1848.
- 33 Yaturu S, Daberry RP, Rains J, Jain S. Resistin and adiponectin levels in subjects with coronary artery disease and type 2 diabetes. *Cytokine* 2006; **34**:219–223.
- 34 Koch A, Gressner OA, Sanson E, et al. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of nonseptic patients. *Crit Care* 2009; **13**:1–9.
- 35 Takeishi Y, Niizeki T, Arimoto T, et al. Serum resistin is associated with high risk in patients with congestive heart failure: a novel link between metabolic signals and heart failure. *Circ J* 2007; **71**:460–464.
- 36 Ellington AA, Malik AR, Klee GG, et al. Association of plasma resistin with glomerular filtration rate and albuminuria in hypertensive adults. *Hypertension* 2007; **50**:708–714.
- 37 Axelsson J, Bergsten A, Qureshi AR, et al. Elevated resistin levels in chronic kidney disease are associated with decreased glomerular filtration rate and inflammation, but not with insulin resistance. *Kidney Int* 2006; **69**:596–604.
- 38 D'Apolito M, Du X, Zong H, et al. Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. *J Clin Invest* 2010; **120**:203–213.
- 39 Graveleau C, Zaha VG, Mohajer A, et al. Mouse and human resistins impair glucose transport in primary mouse cardiomyocytes, and oligomerization is required for this biological action. *J Biol Chem* 2005; **280**:31679–31685.
- 40 Cohen G, Ilic D, Raupachova J, Horl WH. Resistin inhibits essential functions of polymorphonuclear leukocytes. *J Immunol* 2008; **181**:3761–3768.
- 41 Fildes JE, Shaw SM, Yonan N, Williams SG. The immune system and chronic heart failure: is the heart in control? *J Am Coll Cardiol* 2009; **53**:1013–1020.
- 42 Vredoevoe DL, Woo MA, Doering LV, et al. Skin test anergy in advanced heart failure secondary to either ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 1998; **82**:323–328.