Serum resistin is related to plasma HDL cholesterol and inversely correlated with LDL cholesterol in diabetic and obese humans

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Abstract

Objectives: Plasma cholesterol, triglycerides and serum resistin may all be influenced by diabetes and obesity, but their associations remain unclear. Therefore, we put forward a hypothesis that serum lipids might be parallel to resistin, as they all reflect the metabolic status of obese humans.

Design and setting: We measured the concentrations of resistin, total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglycerides (TG) in 134 obese non-diabetic (73 women and 61 men) and 65 obese diabetic (33 women, 32 men) humans, and examined their interrelations. Obesity was defined according to the WHO criterion (BMI, ≥30 kg/m2). The presence of diabetes was the only differentiating factor between two groups of frankly obese humans.

Results: Non-diabetic vs. diabetic, median and interquartile range, respectively:
- Resistin (ng/mL): 26.08, 16.09 vs. 22.37, 14.54, p=0.736
- TC (mmol/L): 5.02, 1.39 vs. 5.16, 1.56, p=0.374
- HDL-C (mmol/L): 1.10, 0.41 vs. 1.02, 0.47, p<0.05
- LDL-C (mmol/L): 3.00, 1.05 vs. 3.00, 1.30, p=0.978
- TG (mmol/L): 1.70, 1.43 vs. 1.95, 1.81, p<0.05

To investigate the interrelations between resistin and lipids, a simple regression analysis was used, and the results were for resistin & TC, HDL-C, LDL-C, and TG, respectively: in the whole cohort r=–0.1364, p=0.0670, r=0.1514, p=0.0437, r=−0.2573, p=0.0006, r=−0.0434, p=0.5597; in non-diabetics: r=−0.2067, p=0.0213, r=−0.1023, p=0.2621, r=−0.2399, p=0.0083 and r=0.0288, p=0.7497; in diabetics r=0.0280, p=0.8360, r=0.2267, p=0.0929, r=−0.2933, p=0.0298, r=0.1349, p=0.3127.

Conclusions: In diabetic and non-diabetic subjects the atherogenic LDL cholesterol shows an inverse correlation with resistin, whereas the protective anti-atherosclerotic HDL cholesterol is positively correlated with resistin.

Abbreviations:
- TC - total cholesterol
- HDL-C - high density lipoprotein cholesterol
- LDL-C - low density lipoprotein cholesterol
- TG - triglycerides
- BMI - the body mass index
INtRODUCTIoN

Adipose tissue is a complicated endocrine organ with numerous neuroendocrine regulatory pathways, and it plays an important role in the pathogenesis of obesity and type 2 diabetes mellitus. Endocrine substances, or adipokines, secreted by this tissue include adiponectin, leptin, IL-6, TNFα (Spiegelman and Flier 1996; Friedman and Halaas 1998; Prins 2002; Steppan and Lazar 2002), and resistin (FIZZ3/ADSF). The latter belongs to the family of cysteine-rich resistin-like molecules (RELM) (Steppan and Brown et al. 2001), together with RELM-α and RELM-β. Serum concentrations of resistin are increased in obese mice and influence insulin action (Steppan and Bailey et al. 2001), however, although resistin might link obesity with insulin resistance and diabetes in mice models, numerous studies in rodent models have called this interesting idea into question (Juan et al. 2001; Le Lay et al. 2001; Way et al. 2001). In humans, the role of resistin is even more controversial. In contrast to mice, human resistin is barely detectable in adipose tissue (Nagaev and Smith 2001; Savage et al. 2001), and no correlation was found between resistin expression of isolated adipocytes and obesity or type 2 diabetes (Nagaev and Smith 2001; Savage et al. 2001; Janke et al. 2002). Furthermore, serum resistin levels were found to be related to body mass index (BMI) in human subjects by some authors (Azuma et al. 2003; Degawa-Yamauchi et al. 2003; Fujinami et al. 2004), whereas others did not demonstrate this association (Heilbronn et al. 2004; Mcternan et al. 2003; Youn et al. 2004).

As plasma cholesterol and triglycerides influence the metabolic status and are important risk factors for cardiovascular damage in obese and diabetic humans, we hypothesized that resistin levels could be related to plasma lipids. Both plasma lipids and resistin are substances which may be influenced by diabetes and obesity. Therefore, the aim of our study was to measure resistin concentrations in non-diabetic and diabetic humans with a similar range of trunkal obesity, and to examine whether resistin concentrations were related to the levels of plasma cholesterol and triglycerides. For this purpose, we investigated two homogenous groups of obese humans, in whom the presence of type 2 diabetes mellitus was the solely differentiating factor.

SUBJECTS AND METHODS

The study group consisted of 134 obese non-diabetic (73 women and 61 men) and 65 obese diabetic (33 women, 32 men) humans recruited by local advertising. Their ages were respectively: 50, 14 years; and 55, 12 years (data shown as median and inter-quartile range). Obesity was defined according to the WHO criterion (BMI, ≥30 kg/m²). All diabetic and non-diabetic subjects suffered from hypertension, well controlled on medication with ACE-inhibitors. Except for hypertension, all subjects were otherwise healthy according to history, clinical examination, and routine laboratory findings. In particular, none of the studied subjects had evidence of acute or chronic inflammatory disease, had a history of alcohol overconsumption, was completely sedentary, or involved in athletics. In all non-diabetics, a normal glucose tolerance was confirmed with the standard oral glucose tolerance test: 75 grams of glucose was administered orally and the plasma glucose concentration was measured in 2 hours. In the diabetes group, all subjects presented with type 2 diabetes mellitus. Diabetes was defined according to WHO criterion (fasting glucose equal or more than 126 mg/dL, or 6.99 mmol/L, on two various days). All subjects were treated with oral sulfonylureas and diabetes was controlled sufficiently: fasting plasma glucose close to the values found in the non-diabetic subgroup was the condition of entering into the study.

All subjects were examined in the morning (at 08:00 a.m.) after an overnight fast. Waist and hip circumferences where measured. Repeated bioimpedance tests were performed at the same time after an overnight fast (08:00 a.m.). Exercise, and alcohol or caffeine consumption was banned 24 hrs prior to the bioimpedance test. A venous blood sample was obtained for the measurement of plasma levels of glucose, total plasma cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) at the hospital’s routine chemistry laboratory. Serum levels of resistin were measured using an ELISA method (R&D Systems, Inc., Minneapolis, MN) and were expressed as nanograms per milliliter. Resistin was measured according to the manufacturer’s recommended protocol, with a sensitivity of 0.026 ng/mL. An intra-assay and inter-assay coefficients of variation were 5.25% and 6.47%, respectively.

The values are given as the median and inter-quartile range. Statistical tests included Mann-Whitney test, Kruskal-Wallis test, Dunn test (for post hoc analysis), and Spearman correlation analysis. STATISTICA 6.0 (StatSoft, Inc., U.S.A.) software was used for statistical analyses.

The study was approved by the ethics committee of the Poznań University of Medical Sciences. All subjects gave informed consent to participate.

RESULTS

The clinical data of diabetic and non-diabetic subjects are shown in Table 1, both genders, and women and men separately. Diabetic patients showed higher levels of glycosylated haemoglobin, plasma glucose and TG, and lower HDL-C. Except for that, the study groups were of similar age, and their anthropometric characteristics, percent body fat, as well as serum resistin concentrations did not differ. For both sexes compared separately, no differences were found, except plasma glucose and HbA1c (Table 1).

To investigate the influence of resistin on serum concentrations of HDL-C, LDL-C, TC and TG, correla-
tions between those lipids and resistin were calculated as follows: for all subjects grouped together, for diabetics only and for non-diabetics only. In order to evaluate the influence of sex, we also estimated all correlations for females and males separately.

In a simple regression analysis performed in the whole cohort, serum levels of resistin were correlated with the levels of HDL-C and LDL-C (r = 0.1514; p = 0.0437, and r = -0.2573; p = 0.0006, respectively), but did not correlate with TC and TG (r = -0.1364; p = 0.0670, and r = 0.0434; p = 0.5597, respectively). Dissimilar results were found in non-diabetics: r = -0.2067; p = 0.0213, r = 0.1023; p = 0.2621, r = -0.2399; p = 0.0083 and r = -0.3050; p = 0.0199, for TC, HDL-C, LDL-C and TG, respectively. In diabetics, serum resistin did not correlate with TC and TG (r = -0.0079; p = 0.9676, r = -0.2138; p = 0.2565, r = -0.3606; p = 0.0546, and r = -0.1800; p = 0.3413; in non-diabetic men: r = -0.3050; p = 0.0199, r = -0.0227; p = 0.8667, r = -0.2172; p = 0.1112, and r = -0.0898; p = 0.5027. Furthermore, in all men assessed together, serum levels of resistin were not correlated with TC and TG (r = -0.0658; p = 0.5287, r = 0.0714; p = 0.4915, respectively). In contrast, resistin correlated with HDL-C and inversely with LDL-C (r = 0.2624; p = 0.0120, r = -0.2458; p = 0.0189, respectively) in this group.

**Women.** In diabetic women, serum levels of resistin did not correlate with the levels of TC, HDL-C, LDL-C and TG (r = 0.0211; p = 0.9152, r = 0.2901; p = 0.1506, and r = -0.1164; p = 0.5711, r = -0.0120; p = 0.9515, respectively). In non-diabetic women, serum resistin inversely correlated with LDL-C levels (r = -0.2838; p = 0.0220), whereas it did not correlate with other lipids (r = -0.1005; p = 0.4219, r = 0.1853; p = 0.1395, and r = 0.1412; p = 0.2545, for TC, HDL-C and TG, respectively). In all women assessed together, serum levels of resistin were not correlated with TC and TG (r = -0.0658; p = 0.5287, r = 0.0714; p = 0.4915, respectively). In contrast, resistin correlated with HDL-C and inversely with LDL-C (r = 0.2624; p = 0.0120, r = -0.2458; p = 0.0189, respectively) in this group.

**Men.** The results for TC, HDL-C, LDL-C and TG were respectively: in diabetic men: r = -0.0797; p = 0.9676, r = 0.2138; p = 0.2565, r = -0.3606; p = 0.0546, and r = -0.1800; p = 0.3413; in non-diabetic men: r = -0.3050; p = 0.0199, r = -0.0227; p = 0.8667, r = -0.2172; p = 0.1112, and r = -0.0898; p = 0.5027. Furthermore, in all men assessed together resistin levels were not correlated with the levels of TC, HDL-C, TG, but they inversely correlated with LDL-C (r = -0.1991; p = 0.0645, r = -0.0772; p = -0.4773, r = -0.0053; p = 0.9606, and r = 0.2458; p = 0.0189, respectively).

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For the correlation analysis, the significance level was set to p < 0.05.

**Tab 1.** Clinical characteristics of non-diabetic and diabetic subjects examined. Data are shown as median and inter-quartile range, and presented separately for women, separately for men, and for both genders together.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetics</th>
<th></th>
<th>Diabetics</th>
<th></th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
<td>Both genders</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Number</td>
<td>73</td>
<td>61</td>
<td>134</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>37.30; 33.10</td>
<td>10.40; 9.10</td>
<td>35.35; 10.30</td>
<td>36.30; 34.05</td>
<td>7.40</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>110.5; 112.0</td>
<td>21.50; 24.0</td>
<td>111.0; 20.0</td>
<td>111.0; 116.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>123.5; 110.0</td>
<td>21.50; 22.0</td>
<td>117.0; 22.0</td>
<td>120.0; 115.5</td>
<td>20.0</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>5.4; 6.60</td>
<td>1.10; 0.80</td>
<td>5.50; 0.90</td>
<td>7.73; 6.70</td>
<td>3.07</td>
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<td>HbA1c (mmol/L)</td>
<td>5.9; 5.9</td>
<td>0.90; 0.80</td>
<td>5.90; 0.90</td>
<td>7.45; 7.25</td>
<td>2.25</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.93; 5.28</td>
<td>0.98; 1.88</td>
<td>5.02; 1.39</td>
<td>5.18; 5.12</td>
<td>1.13</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.17; 1.01</td>
<td>0.40; 0.36</td>
<td>1.10; 0.41</td>
<td>1.13; 0.94</td>
<td>0.58</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.0; 3.00</td>
<td>0.70; 1.10</td>
<td>3.00; 1.05</td>
<td>3.45; 2.74</td>
<td>1.30</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.36; 2.18</td>
<td>1.02; 1.72</td>
<td>1.70; 1.43</td>
<td>1.72; 2.54</td>
<td>1.25</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>26.88; 25.47</td>
<td>15.25; 16.86</td>
<td>26.08; 16.09</td>
<td>20.75; 27.43</td>
<td>15.69</td>
</tr>
</tbody>
</table>

*p-value* for comparisons: Women vs. Men: <0.001, Both genders: <0.0001.
DISCUSSION

Findings from animal model studies suggested that resistin could be an important factor in the development of obesity and type 2 diabetes (Steppan and Bailey et al. 2001; Pang and Le 2006; Muse et al. 2007). However, the link between resistin and obesity in humans is less clear, and conflicting results were reported. Most of the human studies failed to show this link (Nagaev et al. 2001; Savage et al. 2001; Patel et al. 2003), and only a few studies demonstrated that resistin serum concentrations were elevated in obese patients and could be related to obesity (McTernan PG et al. 2002; McTernan CL et al. 2002).

Even less is known about the relationships between resistin and plasma lipids, which prompted us to investigate this problem. Since plasma lipids are a strong risk factor for the development of atherosclerosis which is the major cause of cardiovascular system damage in diabetes and obesity, it seemed logical to us that the serum resistin might be parallel to plasma lipids. These parallel concentrations occurred indeed, unexpectedly however, what we demonstrated in this study was that in both diabetic and non-diabetic subjects the atherogenic LDL cholesterol showed an inverse correlation with resistin, whereas the protective anti-atheroscle-
rotic HDL cholesterol was positively correlated with resistin. This is in contrast to what found Osawa et al. (2007). Furthermore, a correlation between total cholesterol and resistin was found only in the whole cohort, and disappeared in separated groups. The fact that LDL cholesterol was inversely correlated with resistin seemed surprising at first sight. Indeed, we expected a positive correlation as both resistin and LDL cholesterol are known as atherosclerosis risk factors. Resistin mRNA and protein were demonstrated in atherosclerotic lesions in the aorta of mice, and resistin showed a relationship with coronary heart disease in cross-sectional studies (Burnett et al. 2005; Reilly et al. 2005; Ohmori et al. 2005). The explanation of this inverse correlation might be difficult, particularly because the sources of resistin differ between rodents and humans. The adipocyte is presumably the sole source of resistin in mice, whereas in humans the majority of resistin is secreted by macrophages and monocytes, and only little is produced by adipocytes. Obviously, our clinical study was not aimed at the explanation of this unexpectedly inverse correlation, and further research is necessary. Still, we might propose some theoretical explanation: presumably, a high LDL cholesterol concentration inhibits resistin secretion by the macrophages and monocytes. The fact that resistin molecules were found in atherosclerotic lesions, as were LDL-C molecules, might point to some competitive mechanisms between LDL-C and resistin. This hypothesis necessitates further research.

In addition, we would like to comment on the approach we used in this study. Here we investigated two groups of obese patients, in whom the presence of type 2 diabetes was the only differentiating factor. However, we decided to examine only diabetic subjects with a correct, or nearly correct fasting plasma glucose achieved on medication. One may argue about this limitation, which is in contrast to many other studies. This approach diminished our sample but gave us the opportunity to rule out the influence of imbalanced diabetes on the secretion of resistin. In other words, we analyzed a strictly limited group of obese diabetic and euglycemic individuals, with the diabetic subgroup well balanced on medication. Also, because gender may be a confounding factor, we studied both men and women, and evaluated them separately. Including only women and only men into the analyses yielded comparable results as including all individuals.

Based on our results, we show here that resistin levels are paradoxically correlated with the plasma LDL and HDL cholesterol levels in obese humans. Importantly, this correlation occurs for LDL cholesterol both in diabetic and non-diabetic obese patients. The findings from our study may be another piece of information to the discussion about the role of resistin in human metabolism.

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