EXPERIMENTAL STUDY

Serum Reg-Iα is not Suitable Marker of Metabolic Syndrome

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Abstract

Background: Reg-Iα plays a role in various types of tissue regeneration.
Aim: To evaluate serum Reg-Iα for the diagnosis of metabolic syndrome.
Methods: 14 non-obese, healthy subjects and 15 individuals with metabolic syndrome were studied. Anthropometric and laboratory analysis in sera (Body Mass Index – BMI, insulin, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, uric acid, aglucose, Quicki calculation, Reg-Iα) were performed.
Results: Reg-Iα levels did not differ between subjects with metabolic syndrome and healthy subjects (means 597.7 vs 631.1 ng/l, p<0.01) and positively correlated only with fasting glucose (r=0.34; p<0.01) and age (r=0.38; p<0.01); Reg-Iα correlated even after adjustment to age. Reg-Iα concentrations did not differ in men and women.
Conclusions: Our study, for the first time, indicates that serum Reg-Iα is not useful for the diagnosis of metabolic syndrome (Tab. 3, Ref. 7). Full Text (Free, PDF) www.bmj.sk.
Key words: serum Reg-Iα, metabolic syndrome, tissue regeneration.

Metabolic syndrome is constituted from many characters and symptoms (e.g. dyslipidemia, hypertension, impaired glucose tolerance, insulin resistance, obesity, hyperuricemia, trombophlia, atherosclerosis). In the development of metabolic syndrome, the principal role is presumably played by adipose tissue.

Adipose tissue has paracrine, autocrine and endocrine effects and produces many bioactive substances (e.g. adipokines, cytokines, free fatty acids) into circulation. These substances regulate energy metabolism, appetite, insulin sensitivity, aging process, vascular reactivity, vascular smooth muscle cells proliferation and inflammation with the effects in the brain or other tissues.

Reg-Iα could have a high diagnostic validity for the assessment of tissue stress. There is no valid information about Reg-Iα assessment in the diagnosis of metabolic syndrome (1, 4).

The aim of this study was to evaluate assessment of Reg-Iα for the diagnosis of metabolic syndrome in Caucasian population.

Methods

Studied groups of subjects

The study was approved by the ethical commission of the Hospital Sternberk, Czech Republic. A total of 14 non-obese, healthy (BMI<26) individuals and 15 individuals with metabolic syndrome were recruited for the study.

Subjects with metabolic syndrome were diagnosed on the basis of defined criteria (The criteria proposed by the National Education Program’s Adult Treatment Panel III Report (NCEP-ATP)) (7). It is the presence of at least 3 or more of following abnormalities: 1) hypertension: systolic blood pressure >130 mmHg, diastolic blood pressure >85 mmHg, and/or receiving blood pressure lowering drugs; 2) hyperglycemia: fasting serum glucose concentration >6.1 mmol/l and/or receiving glucose-lowering drugs; 3) hypertriglyceridemia: fasting serum triglyceride concentration >1.69 mmol/l; 4) Low HDL: fasting serum HDL concentration <1.04 or 1.29 mmol/l in males and females re-

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Tab. 1. Basic statistical analysis of individuals without metabolic syndrome.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>Median</th>
<th>SD</th>
<th>Normality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg-1α</td>
<td>597.7</td>
<td>550.7</td>
<td>117.0</td>
<td>yes</td>
</tr>
<tr>
<td>CRP</td>
<td>1.6</td>
<td>0.5</td>
<td>2.5</td>
<td>no</td>
</tr>
<tr>
<td>Chol</td>
<td>4.9</td>
<td>4.8</td>
<td>0.8</td>
<td>yes</td>
</tr>
<tr>
<td>LDL</td>
<td>2.6</td>
<td>2.6</td>
<td>0.3</td>
<td>yes</td>
</tr>
<tr>
<td>Trigl</td>
<td>1.1</td>
<td>1.2</td>
<td>0.4</td>
<td>yes</td>
</tr>
<tr>
<td>Glyk</td>
<td>5.2</td>
<td>5.3</td>
<td>0.4</td>
<td>yes</td>
</tr>
<tr>
<td>BMI</td>
<td>22.7</td>
<td>23.0</td>
<td>1.6</td>
<td>yes</td>
</tr>
<tr>
<td>Age</td>
<td>58.9</td>
<td>57.0</td>
<td>10.5</td>
<td>yes</td>
</tr>
<tr>
<td>Quicki</td>
<td>0.59</td>
<td>0.51</td>
<td>0.23</td>
<td>no</td>
</tr>
</tbody>
</table>

Note: Reg/Age – adjustment of Reg-1α to age of individuals

Sampling and measured data

Anthropometric, clinical and laboratory fasting analysis (height, weight, body mass index, waist circumference, systolic and diastolic pressures) were performed.

Blood samples were drawn under aseptic precautions from a venous cubit, after several minutes of the person’s rest in half-sitting position. Serum sample was separated in a cooled centrifuge at 4 °C at 3000 g and subsequently frozen at -80 °C.

Insulin (DPC, USA), total cholesterol, HDL-cholesterol, LDL-cholesterol (Biovendor Laboratory Medicine, Brno, Czech Republic), triglycerides (Biovendor Laboratory Medicine, Brno, Czech Republic), glucose and uric acid (Biovendor Laboratory Medicine, Brno, Czech Republic) were analysed in fresh sera. Concentrations of Reg-1α were determined after deproteinising the sera during the same day.

The Quicki index was calculated (5, 6).

ELISA for human Reg-1α determination

The ELISA kits were obtained from Biovendor Laboratory Medicine (Brno, Czech Republic). Assay was conducted according to the manufacturer’s instructions. The intra-assay and inter-assay variations were evaluated by measuring 3 different samples in 8 replicates (CV intra-assay <4.9 %, CV inter-assay <8.5 %). In the Biovendor’s Human Reg-1α ELISA, calibrators, quality controls and samples are two polyclonal rabbit anti-human Reg-1α used in sandwich arrangement. The limit of adtection (defined as human Reg-1α concentration giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: A_blank + 3SDblank) is defined as follows:

— Analytical Limit of Detection is calculated from the real Reg-1α values in wells and is 0.18 pg/l.

— Assay Sensitivity considers the dilution of samples and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution = 0.18 Reg-1α x 50 = 9.0 pg/l.

Tab. 2. Basic statistical analysis of individuals with metabolic syndrome.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>Median</th>
<th>SD</th>
<th>Normality</th>
<th>Dif</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg-1α</td>
<td>631.1</td>
<td>442.3</td>
<td>451.7</td>
<td>yes</td>
<td>NS</td>
</tr>
<tr>
<td>CRP</td>
<td>4.3</td>
<td>2.9</td>
<td>4.2</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chol</td>
<td>5.6</td>
<td>5.5</td>
<td>1.0</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL</td>
<td>3.4</td>
<td>3.3</td>
<td>0.8</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Trigl</td>
<td>2.7</td>
<td>2.5</td>
<td>0.8</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glyk</td>
<td>8.6</td>
<td>7.8</td>
<td>2.8</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>35.6</td>
<td>34.7</td>
<td>3.2</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age</td>
<td>61.0</td>
<td>61.0</td>
<td>11.6</td>
<td>yes</td>
<td>NS</td>
</tr>
<tr>
<td>Quicki</td>
<td>0.29</td>
<td>0.29</td>
<td>0.05</td>
<td>yes</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note: Reg/Age – adjustment of Reg-1α to age of individuals

Tab. 3. Significant correlations of Reg-1α.

<table>
<thead>
<tr>
<th>Parameter 1</th>
<th>Parameter 2</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg-1α</td>
<td>Glyk</td>
<td>0.25</td>
<td>0.027</td>
</tr>
<tr>
<td>Reg-1α/Age adj</td>
<td>Glyk</td>
<td>0.23</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The antibodies in Human Reg-1α kit are highly specific for human Reg-1α with no detectable cross-reactivity to human Reg-1α PAP and Reg IV at 50 pg/l. No signal was found to the following animal sera: mouse, goat, hamster, rabbit, horse, sheep, rat, pig, dog and bovine. Spiking Recovery was maximally 94 % and linearity was 92.3 %.

The Human Reg-1α His-Tagged Fusion Protein is used as the acclibrator.

The recombinant Reg-1α is a 17.8 KDa protein containing 144 amino acid residues of the Human Reg-1α and 12 amino acid His-tag. The amino acid sequence of the recombinant Human Reg-1α is 100 % homologous to the amino acid sequence of the Human Reg-1α without signal sequence.

Statistical analysis

Gained data were processed using the software Medcalc (Medcalc, Mariakerke, Belgium). The value p<0.05 was considered statistically significant.

Because there is normal data distribution in evaluated parameters, Pearson’s correlation coefficients were used to establish the association between Reg-1α levels and parameters of metabolic syndrome. Comparison of A Reg-1α values between subjects with and without metabolic syndrome was made using the t test. Stepwise regression and multivariate analysis of markers was performed and selected markers (only age) were used for Reg-1α adjustment. All the data are expressed as medians and means±standard deviations.

Adjustment of Reg-1α for age was proven with regression analysis.
Results

We analysed 29 individuals (18 men, 11 women). 14 of them were healthy (8 men, 6 women) and 15 were subjects with metabolic syndrome (9 men, 6 women).

The circulating concentrations of Reg-1α did not differ in subjects with metabolic syndrome and healthy ones (Tabs 1 and 2) and positively correlated only with fasting serum glucose levels ($r=0.25$; $p=0.03$) and age ($r=0.38$; $p<0.01$) (Tab. 3).

No correlations between Reg-1α and cholesterol, LDL cholesterol, triglycerides, CRP, BMI, blood pressure and Quicki were observed.

Correlations between Reg-1α and serum glucose were present even after Reg-1α adjustment for age ($r=0.23$; $p<0.01$) (Tab. 3).

In both men and women, the circulating concentrations of Reg-1α did not differ in obese and non-obese subjects.

Discussion

Obesity is the most common risk factor for metabolic syndrome which refers to a cluster of abnormalities, including dyslipidemia, insulin resistance, type 2 diabetes, hypertension and atherosclerosis. The molecular pathways are not well understood (1).

Experimental animal and human studies suggest that Reg-1α could be a marker of an expressive tissue stress (2–4).

Since both obesity and metabolic syndrome are considerable stress with relevant tissue complications (e.g. cardiovascular and cerebrovascular diseases, diabetes type 2, hypertension, autonomic vegetative dysfunction), we expect some difference between Reg-1α values in obese individuals with metabolic syndrome compared to healthy individuals.

In 1984, a novel gene was found, which plays a role in various types of tissue regeneration. Recently, the regenerating gene (Reg) has been documented to play an important role in regenerating of myocardium and a transcriptional activation of Reg in the heart in response to heart stress was detected. In damaged human myocardium and some other tissues, Reg-1 gene expression was located in fine granular pattern in the cytoplasm. These results demonstrated the presence of the Reg/Reg receptor system in stressed tissues (2–4). In the view of emerging evidence of Reg for tissue regeneration in a variety of tissues/organs, it is proposed that the tissue may be a target for Reg action and that Reg may protect against acute heart stress.

This study indicated, for the first time, that serum Reg-1α values are not a marker of metabolic syndrome or adiposity. Further research is necessary to confirm the correlations between glycemia and Reg-1α and to evaluate Reg-1α values as a predictor of accelerated atherosclerosis or impaired glucose tolerance.

Competing interests

The authors declare that they have no competing interests.

References


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