Adiponectin, TG/HDL-cholesterol index and hs-CRP. Predictors of insulin resistance.

Bonneau GA\textsuperscript{1,2} y Pedrozo WR\textsuperscript{1}

\textsuperscript{1}Ministry of Public Health, Province of Misiones, \textsuperscript{2}School of Exact, Chemical and Natural Sciences – National University of Misiones

ABSTRACT

The initial disturbance of insulin resistance appears to focus on adipose tissue, which is a dynamic organ involved in many physiological and metabolic processes. It expresses and secretes a variety of active peptides, adipocytokines. The aim of this study was to determine the relationship between TG/HDL-cholesterol index, markers of adipose tissue metabolism (adiponectin) and inflammatory markers (hs-CRP) with insulin resistance in an urban population of the interior of the country. We evaluated 176 employees of 2 public hospitals in Posadas, Misiones, 117 women and 59 men, aged 49.01 ± 9.33 years. The exclusion criteria were diabetes, hypothyroidism or hyperthyroidism, infectious disease, renal or hepatic neoplasms and pregnancy. Blood samples were collected after a 12-hour fasting period. Measurements included: blood glucose and triglycerides by enzymatic colorimetric method according to Trinder, HDL-cholesterol by homogeneous method, insulin and the high-sensitivity C-reactive protein (hs-CRP) by chemiluminescent immunometric assay on a solid phase Immulite 2000-Siemmens, and adiponectin by monoclonal antibodies (ALPCO immunoassays). All samples were processed with calibrators, normal and pathological commercial controls and in-house and external quality controls. The statistical analysis was performed using Epi Info 6.04d. Seventeen point two per cent of the population had IR and 45.7 % had decreased concentrations of adiponectin. IR was significantly associated with Adiponectin (p < 0.001), hs-CRP (p = 0.02) and TG/HDL-cholesterol index (p = 0.02). But the parameter best associated with IR was serum adiponectin concentration. Therefore, we can conclude that the measurement of this hormone can be a useful tool for improving current risk estimates. Rev Argent Endocrinol Metab 50:78-83, 2013

INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide. In Argentina, in 2003, out of a total of 302,064 deaths, 95,090 were from cardiovascular conditions \textsuperscript{(1)}. In the province of Misiones, according to 2011 data provided by the Ministry of Public Health, cardiovascular disease is the main cause of mortality, with a proportional mortality ratio of 28.3 %, and with atherosclerosis as the main responsible factor \textsuperscript{(2)}. At present, atherosclerosis is considered as a chronic inflammatory process where endothelial dysfunction plays a major role. There are many risk factors involved in the silent development of atherosclerosis over time, which have been well defined and studied \textsuperscript{(3)}.

Insulin resistance (IR) promotes accelerated atherosclerotic development and it may be defined as a decreased responsiveness or sensitivity of insulin effectors to promote normal glucose uptake by cells \textsuperscript{(4)}. Resistance to the action of insulin is compensated by an increased secretion of this hormone by β-cell, leading to what is known as “compensatory hyperinsulinemia” to maintain adequate blood glucose levels \textsuperscript{(5)}. Its presence associated with other risk factors is a widely accepted predictor of type 2 diabetes, and even in non-diabetic individuals it is associated with a higher risk for cardiovascular disease \textsuperscript{(4)}.
Plasma triglyceride (TG) and high-density lipoprotein cholesterol (HDL-chol) concentrations are independently associated with the use of insulin-mediated glucose. The TG/HDL-chol index is significantly related with this estimate of insulin action, a widely surrogate estimate of IR (6). As TG and HDL-cholesterol measurements are standardized, while there is no standard method for measuring plasma insulin concentration, this index could be a very useful indirect estimate for assessing IR in intermediate risk for cardiovascular disease. Even if evidence suggests that the mere presence of elevated levels of RCP would increase the likelihood of cardiovascular disease, the role of RCP as an independent risk factor remains controversial. A large number of methods are available at the clinical lab for measuring reactive C protein. For CRP to be useful in predicting cardiovascular disease, the so called “ultrasensitive” methods are required, which may detect concentrations between 0.15 and 20 mg/L. C reactive protein measured by these techniques is known as hs-CRP (high sensitivity C-reactive protein) (8).

The initial disturbance of IR appears to focus on adipocytes and it consists in an inability to store fatty acids. This inability would be secondary to genetic predisposition and diet alterations, among other factors and, in this context, the adipocyte may be considered as a secreting endocrine organ, as the "dysfunctional adipocyte" secrets a large number of biologically active proteins and peptides involved in energy homeostasis and in the regulation of neuroendocrine and immunological functions, which are known as adipokines (9).

Adipokines act in an autocrine, paracrine or endocrine fashion to regulate several metabolic functions, many of them involved in the pathogenesis of IR (10).

Adiponectin circulates in plasma in concentrations ranging between 5 and 30 µg/mL and account for approximately 0.01 % of total plasma proteins (11).

Since the discovery of adiponectin, a large number of groups have reported a strong negative correlation between circulating levels of adiponectin and IR, both in human and animal species and in in vivo and in vitro studies (12-15).

Reduced levels of adiponectin are observed in a large variety of pathological conditions associated with IR, type 2 diabetes, cardiovascular disease (16-18). However, it is still unknown if low levels of adiponectin are the cause or the consequence of these conditions (11, 19).

The gold standard method for assessing IR is the hyperinsulinemic-euglycemic clamp, but as it is complex to undertake, various mathematical formulas have been developed as an approximation to the clamp. One of the most widely used is the HOMA index, which may be applied with a simple measurement of fasting blood glucose and insulin.

The aim of this study was to determine the relationship between the TG/HDL-chol index, markers of adipose tissue metabolism (adiponectin), inflammatory markers (hs-CRP) and IR en an urban population of the interior of Argentina, represented by employees of two public hospitals in the province of Misiones.

MATERIALS AND METHODS

Population

This study was conducted as part of a project that consisted in a ten-year follow-up of a population of approximately 1000 public employees at two institutions: Hospital Dr. Ramón Madariaga and Hospital de Pediatría Dr. Fernando Barreiro in Posadas (province of Misiones), with the aim of assessing cardiovascular risk factors and associated habits every two years. The study was initiated in 2001, and in 2011 and
2012, we evaluated a sample of 176 individuals from the population that was being followed up. The sample included 117 women and 59 men with ages ranging between 25 and 74 years and a mean ± SD of 49.01 ± 9.33 years.

**Inclusion criteria:** We enrolled subjects who voluntarily agreed to participate in the study.

**Exclusion criteria:** Individuals were excluded if they met any of the following criteria: diabetes, hypothyroidism or hyperthyroidism, infectious, kidney or liver disease, malignancies, pregnant women or subjects on current treatment with lipid-lowering agents.

**Collection of samples**

Blood samples were obtained by venipuncture after a 12-hour fasting period, and were placed in primary separator tubes. Serum was separated in clean and dry tubes for automated analyzer within 2 hours of collection, for biochemical measurements.

**Biochemical parameters**

Serum concentrations of glucose (coefficient of variation [CV] = 2.43%) and triglycerides (CV = 2.45%) were measured by enzymatic colorimetric endpoint method (Trinder). HDL-chol levels (CV = 3.41) were measured by homogeneous method. Based on the latter two biochemical parameters, the TG/HDL-chol index was calculated, considered a value ≥ as increased. Samples were processed in an automated analyzer “Dimension RxL Max clinical chemistry system”, with Siemens equipment and reagents. We used commercially available pathologic and normal control sera and calibrators, and an in-house quality control serum pool prepared at the central laboratory of Hospital Dr. Ramón Madariaga. External Quality Control was carried out with controls provided by the Argentine Biochemical Foundation and CEMIC.

Measurements of hs-CRP (high-sensitivity C-reactive protein) (CV = 2.1%) and insulin (CV = 4.2%) were performed using a solid-phase chemiluminescent immunometric assay (Immuliite 2000, Siemens).

Values of hs-CRP were considered to indicate low risk if < 1 mg/l, intermediate risk between 1 and 3 mg/l and high risk if > 3 mg/l.

With the blood glucose and insulin data we calculated the IR index, HOMA (Homeostasis Model Assessment = fasting insulin (µU/ml) x fasting glucose (mmol/l))/22.5), taking a cut-off value of for IR ≥ 2.6 (20).

Plasma adiponectin levels were assessed by ALISEI v 2.2 (microplate enzyme immunoassay automated analyzer – SEACP SRL - Florence, Italy) using monoclonal antibodies (ALPCO immunoassays) (CV = 3.2%). Cut-off values for adiponectin were: < 5 µg/ml were considered as decreased and values ≥ 5 µg/ml were considered as normal (11).

**Statistical Analysis**

Population distribution was evaluated using the Kolmogorov-Smirnov normality test. Based on this evaluation, medians and percentiles were used for a descriptive analysis of non-parametric variables. The U Mann Whitney test was used for comparison between groups. A multifactorial analysis was performed by logistic regression with the aim of identifying independent variables for IR. Results were analyzed using the statistical software Epi Info 6.04d. All analyses were performed with 95% confidence interval and a significance level set at < 0.05.
Ethical concerns

Subjects were duly informed of their rights as research subjects and confidentiality of data. All subjects signed their consent prior to the initiation of the study, in accordance with the Declaration of Helsinki.

This study was approved by the Teaching and Research Committee and the Bioethics Committee at the two participating hospitals.

Subjects who took part in the study were provided with the results of their tests and received medical care from a general practitioner, cardiologist or endocrinologist, as appropriate. Advice was sought from a nutritionist on diet plans tailored to the needs of each individual. A number of physical activities were scheduled for participants by gym teachers.

RESULTS

We found that 17.2% of all subjects studied had IR and 45.7% had decreased adiponectin concentrations.

Table I. Biochemical characteristics of all 176 study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (P25-P75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>82 (73 – 90)</td>
</tr>
<tr>
<td>Fasting blood insulin (µU/mL)</td>
<td>4.02 (2.01 – 8.40)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.81 (0.43 – 1.84)</td>
</tr>
<tr>
<td>Triglycerides/HDL-chol index</td>
<td>2.46 (1.51 – 3.77)</td>
</tr>
<tr>
<td>Total adiponectin (µg/mL)</td>
<td>5.47 (3.82 – 7.96)</td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>2.40 (3.82 – 7.96)</td>
</tr>
</tbody>
</table>

*References: P25: 25th percentile – P75: 75th percentile*

Table II. Insulin resistance and its relationship with the TG/HDL-chol index, adiponectin and hs-CRP, in hospital employees (n = 176)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (P25 – P75)</th>
<th>U Mann Whitney test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG/HDL-chol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>4.79 (3.10-5.98)</td>
<td>2442</td>
<td>0.020</td>
</tr>
<tr>
<td>IS</td>
<td>2.42 (1.02-3.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>4.15 (2.18-5.97)</td>
<td>1862</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IS</td>
<td>5.87 (4.03-8.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hs-CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>3.30 (2.20-5.70)</td>
<td>2508</td>
<td>0.020</td>
</tr>
<tr>
<td>IS</td>
<td>2.20 (0.80-4.70)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III. Predictors of insulin-resistance found by logistical regression analysis in hospital employees (n = 176)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>B</th>
<th>SD</th>
<th>Statistical</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-14.78</td>
<td>3.558</td>
<td>17.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total adiponectin</td>
<td>-3.68</td>
<td>0.115</td>
<td>9.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>-0.067</td>
<td>0.441</td>
<td>0.44</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides/HDL-chol index</td>
<td>0.015</td>
<td>0.032</td>
<td>0.24</td>
<td>NS</td>
</tr>
</tbody>
</table>

DISCUSSION

Current risk scales have limitations for predicting the likelihood for cardiovascular events. For a simple identification of individuals with insulin resistance and at higher risk of developing cardiac-metabolic disease in relation with IR, the TG/HDL-chol index has been proposed. This is a very simple calculation, of low cost, acceptable sensitivity and specificity, performed with routine tests. For this reason, this parameter was included in our study and we found an association with IR in our hospital employees.

It is important to highlight that a highly significant association was found between serum concentrations of adiponectin and IR, which is consistent with previous studies, where it is emphasized that as from the discovery of this hormone, a large number of research groups have reported the existence of a strong negative correlation between circulating levels of adiponectin and IR, both in human subjects and in animal species, and in in vivo and in vitro studies (11-13). It is still unknown if low levels of adiponectin are the cause or the consequence of these conditions. It is interesting to speculate that genetic variation may decrease circulating levels of adiponectin and therefore predispose to IR, but it has also been observed that when IR has developed, suppression of circulating levels of adiponectin is enhanced, and this could be due to genetic susceptibility to IR conferred by the ADIPOQ gene polymorphisms (9, 11).

Further research into these possibilities remains to be done but at least, evidence to date shows that the decrease in adiponectin concentrations is common in IR.

Although circulating hs-CRP is an inflammatory marker, its value as a predictor of cardiovascular events has not been confirmed and there are many controversies on this issue (8, 21-23). When levels of hs-CRP were evaluated in our population, a significant association with IR was found.

Nevertheless, when all variables were independently assessed in order to identify which could best account for IR or was a better predictor of the event, only serum concentrations of adiponectin were found to have an association.

Some studies (23, 14) show that low adiponectin levels are associated with a higher risk of cardiovascular events, but only in subjects with IR where adiponectin plays an important role in its physiopathology. In addition, the REFERENCE study (23) found that 35% of patients who had experienced a cardiovascular event had high risk score; therefore, finding other measurements that might help to predict events remains a challenge.

Scientific data reported to date by various research groups support the idea that adiponectin is a useful resource for improving risk prediction.
CONCLUSIONS

In the population studied, the parameter that was found to be best associated with IR was serum adiponectin concentration. This is of note if we consider that one in five individuals that participated in the study had insulin resistance according to the HOMA-IR index.

Acknowledgments: We would like to thank Siemens Healthcare for donating reagents, the Alberto J. Roemmers foundation for providing grants for the purchase of reagent, the hospital employees for their willingness to participate in this study and the GEIFRM group for their participation in data collection.

REFERENCES


