A comparison of osteoprotegerin with adiponectin and high-sensitivity C-reactive protein (hsCRP) as a marker for insulin resistance


Objective. Insulin resistance (IR) is associated with low adiponectin and elevated high sensitivity C-reactive protein (hsCRP). Osteoprotegerin (OPG) has been shown to be elevated in type 2 diabetes, but whether it reflects underlying IR is unclear. We aimed to compare the ability of serum OPG with adiponectin and hsCRP to act as a marker for IR in individuals with normal and abnormal glucose tolerance.

Materials/methods. 115 men underwent a 75 g oral glucose tolerance test. OPG, hsCRP and adiponectin were measured using ELISA. IR was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR).

Results. Men with abnormal glucose tolerance (n=38) were older (58.3±11.2 vs 47.3±11.4 years, P<.001), had higher body mass index (BMI) (31.1±2.9 vs 27.9±3.2 kg/m², P<.001) and were more insulin resistant (median (I.Q.) HOMA-IR 5.88 (3.38) vs 1.13 (1.14), P<.001) than those with normal glucose tolerance (n=77). After adjustment for age and BMI, OPG (6.28 (2.32) vs 5.16 (1.86) pmol/L, P<.001) and hsCRP (2.07 (5.47) vs 0.78 (1.05) mg/L, P<.001) were higher and adiponectin (3.02±1.17 vs 4.78±2.38 μg/mL, P<.001) was lower in those with AGT. After adjustment for age and BMI, adiponectin (r = −0.317, P<.001) and hsCRP (r = 0.318, P<.001), but not OPG (r = 0.126, P = .196) correlated with HOMA-IR. On multiple linear regression analysis, adiponectin and hsCRP but not OPG were independent predictors of HOMA-IR.

Conclusions. OPG is higher in individuals with abnormal glucose tolerance, but unlike adiponectin and hsCRP, does not correlate with HOMA-IR, suggesting its elevation within this cohort of individuals is due to factors other than insulin resistance.

Abbreviations: hsCRP, high sensitivity C-reactive protein; OPG, Osteoprotegerin; HOMA-IR, homeostatic model assessment of insulin resistance; IR, insulin resistance; NGT, normal glucose; OGTT, oral glucose tolerance test; AUC, Area under the curve; ACE, angiotensin converting enzyme; ARB, inhibitor, angiotensin receptor blocker.

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1. Introduction

Insulin resistance (IR) is a metabolic state associated with increased risk of type 2 diabetes and cardiovascular disease through a variety of different molecular mechanisms [1]. It is an inflammatory state characterised by elevated levels of pro-inflammatory cytokines such as high sensitivity C-reactive protein (hsCRP) [2] and low levels of the anti-inflammatory, anti-atherogenic adipocytokine adiponectin [3].

Osteoprotegerin (OPG) is a glycoprotein which was originally identified as an anti-resorptive agent in bone [4,5]. Subsequently, elevated levels were found in individuals with cardiovascular disease [6] and type 2 diabetes [7,8]. Although IR is a key feature of the cardiometabolic syndrome in which diabetes and cardiovascular disease often co-exist, the relationship between serum OPG and IR remains unclear. A positive correlation between OPG and hsCRP has been demonstrated in a number of studies [9,10], but inconsistent relationships between OPG and adiponectin have been described.

The aims of our study were to measure OPG levels in a cohort of individuals with normal glucose (NGT) and glucose intolerance either in the form of impaired fasting glucose, impaired glucose tolerance or newly diagnosed type 2 diabetes; evaluating the correlation between OPG and IR and comparing the findings to the well-documented relationships of adiponectin and hsCRP with IR. In order to control for potential different rates of bone turnover between the two groups, we measured a serum marker of bone formation (osteocalcin) and a marker of bone resorption (serum C-telopeptide of type 1 collagen — CTX).

2. Subjects and Methods

2.1. Subjects

A total of 115 men were consecutively recruited from the diabetes screening programme in the diabetes department in Beaumont Hospital, Dublin and from a study of metabolic parameters of healthy individuals in Dublin City University. Patients who had a current or history of malignancy, renal impairment, previous diagnosis of diabetes, any disorder of calcium metabolism, previous diagnosis of osteoporosis or use of medications affecting bone metabolism, recent (within previous 6 months) history of a macrovascular event (acute coronary syndrome/cerebrovascular event/lower limb embolic event or vascular intervention) or a recent fracture were excluded from the study. The sample size of 115 subjects provided a 92% power to detect a moderate correlation of \( r=0.3 \) between OPG and HOMA-IR, with an \( \alpha \) of 0.05.

Approval was obtained from the Research Ethics Committees at Beaumont Hospital and Dublin City University and all participants provided informed written consent.

2.2. Experimental procedures

All participants underwent a full clinical history and physical examination. Blood was drawn between 08.00 and 09.00 after an overnight fast. Patients underwent a 75 g 2 h oral glucose tolerance test (OGTT), in which a 75 g glucose drink was consumed by the patient and samples for glucose and insulin were taken at 30, 60, 90 and 120 min. WHO criteria were used to diagnose impaired glucose tolerance (IGT) and type 2 diabetes [11]. Area under the curve (AUC) for glucose and insulin was determined by the trapezoidal method. IR was calculated with the homeostatic model assessment of insulin resistance (HOMA-IR) using fasting insulin (mU/L)×glucose (mmol/L)/22.5 [12].

2.3. Biochemical assays

Serum samples were centrifuged at 3000 rpm for 15 min and stored at \(-80^\circ\)C for later analysis of OPG, adiponectin and hsCRP. OPG was measured using commercial enzyme-linked immunosorbent assay kits. Total OPG (Biomedica, Vienna; catalogue no. BI-20402) — i.e. that bound to Receptor Activator for Nuclear Factor Kappa beta Ligand (RANKL) and free in the serum — had intra- and inter-assay variations of <6%, with a minimal detection limit of 0.014 pmol/L. Total (i.e. low and high molecular weight) adiponectin (R&D Systems, Minneapolis, USA; catalogue number DRP300) had intra- and inter-assay variations of <5%, and a minimal detection limit of 0.24 6 ng/mL. Osteocalcin and serum CTX were measured by electrochemiluminescence immunoassay “ECLIA”s on the Roche Elecsys 2010 analyser with minimal detection limits of 0.5 ng/mL and 0.01 ng/mL respectively; inter- and intra-assay coefficients of variation for both assays were <5.5% and <7.5% respectively. Measurement of hsCRP was carried out using Randox reagents, on the Randox Daytona (Randox, Antrim, Northern Ireland). All analyses were performed in duplicate and the average of the duplicated readings used. If a co-efficient of variation of >12% was noted between 2 duplicated samples, repeat analysis was performed.

2.4. Statistical analysis

Categorical variables were reported as frequencies (%) and continuous variables were reported using mean±standard deviation (SD). The variables with abnormal distribution (fasting glucose, fasting insulin, AUC glucose, AUC insulin, HOMA-IR, HDL, triglycerides and hsCRP) were log-transformed and presented as median (interquartile range). A Mann Whitney U test was used to compare differences between the means. Partial correlations were performed using log-transformed values, controlling for age and BMI. Multiple linear regression analysis was performed to identify independent predictors of HOMA-IR using recognised clinical parameters associated with IR. Statistical analysis was carried out using SPSS 17.0 for Windows (SPSS Inc., USA).

3. Results

There were 77 individuals with NGT, 18 with IGT and 20 with newly-diagnosed type 2 diabetes. As there was no difference in IR (or in fact any other parameter except younger age (53.95±2.56 vs 63.47±2.07 years, \( P=.008 \)) and lower AUC for insulin (8864.32±1239.40 vs 12593.67±1304.76, \( P=.036 \)) in those with diabetes compared to those with IGT, we grouped these individuals together to form a cohort of “abnormal glucose
4. Discussion

We found OPG to be higher in individuals with AGT when compared to normoglycaemic healthy controls. However OPG did not correlate with the degree of underlying IR as measured by HOMA-IR either on univariate or multiple linear regression — unlike more well-established markers of dysglycaemia adiponectin and hsCRP.

There have also been conflicting reports of potential relationships between serum OPG and underlying insulin resistance, with both positive and negative associations having been described. The absence of a significant relationship between OPG and HOMA-IR is in contrast to a number of other studies [10,13-15] which consisted of patients with relatively high levels of IR — including patients with type 2 diabetes [13,14] or a previous history of gestational diabetes [10]. In a group of 106 obese patients (18 with type 2 diabetes), Gannage-Yared et al. found a positive association between OPG and HOMA-IR (r = 0.295, P < .01) [13]. In a study by Yaturu et al. [14] there was a significant but weak positive correlation between OPG and HOMA-IR (r = 0.22, P < .05). Akinci et al. found higher OPG in women with a history of gestational diabetes mellitus (GDM) and current metabolic syndrome (MS) but not diabetes compared to those with a history of GDM but no current MS and normal controls [10]. In the group as a whole, there was a weak but statistically significant relationship between OPG and HOMA-IR (r = 0.146, P = .04). Pepene et al. found a positive correlation between OPG and HOMA-IR (r = 0.311, P = .021) in a group of women of whom three quarters had polycystic ovarian syndrome — a condition strongly associated with IR [15].

There have also been a number of studies which have demonstrated a negative association between OPG and IR, but these were predominantly in healthy populations. Our group has previously shown in a rigorously-defined healthy population (all had a normal OGTT and exercise stress test to exclude dysglycaemia and ischaemic heart disease respectively) that OPG correlates negatively with HOMA-IR (r = −0.222, P < .05), suggesting that increasing insulin resistance in a healthy population is associated with lower serum OPG [16]. Other papers which are in agreement with this finding of a negative association between OPG and IR were published by Uğur-Altun and colleagues in 2 separate studies [17,18]. In a complexity-designed study of 50 obese and 24 lean healthy patients in their early thirties without diabetes, OPG correlated negatively with IR (r = −0.428, P = .0001) [17]. The same group later replicated their findings in a group of healthy young women (mean age 31 years) where they again found negative correlations between OPG and fasting insulin (r =
### Table 2 – Partial and bivariate correlations between OPG, adiponectin and hsCRP, and selected clinical and biochemical parameters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adiponectin adjusted for age and BMI</th>
<th>Adiponectin adjusted for age and BMI</th>
<th>hscRP adjusted for age and BMI</th>
<th>hscRP $\pm$ adjusted for age and BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.089</td>
<td>0.365</td>
<td>0.037</td>
<td>0.836</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>-0.142</td>
<td>0.153</td>
<td>-0.164</td>
<td>0.153</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.184</td>
<td>0.058</td>
<td>0.198</td>
<td>0.045</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.020</td>
<td>0.836</td>
<td>0.164</td>
<td>0.099</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>0.062</td>
<td>0.516</td>
<td>0.034</td>
<td>0.730</td>
</tr>
<tr>
<td>AUC glucose (mmol/L/min)</td>
<td>0.116</td>
<td>0.227</td>
<td>0.076</td>
<td>0.436</td>
</tr>
<tr>
<td>Fasting insulin (IU/mL)</td>
<td>0.149</td>
<td>0.117</td>
<td>0.134</td>
<td>0.167</td>
</tr>
<tr>
<td>AUC insulin (IU/mL/min)</td>
<td>0.144</td>
<td>0.132</td>
<td>0.160</td>
<td>0.246</td>
</tr>
<tr>
<td>Quicki</td>
<td>0.103</td>
<td>0.283</td>
<td>0.136</td>
<td>0.167</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>-0.109</td>
<td>0.283</td>
<td>-0.139</td>
<td>0.167</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>-0.109</td>
<td>0.283</td>
<td>-0.139</td>
<td>0.167</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.100</td>
<td>0.369</td>
<td>0.098</td>
<td>0.371</td>
</tr>
</tbody>
</table>

In conclusion, we have shown that although OPG is higher in individuals with abnormal glucose tolerance, this does not appear to be explained by an association with underlying insulin resistance per se. Additionally, we did not find any conclusive evidence of a relationship between OPG and other biomarkers associated with IR namely adiponectin and hsCRP. It is important to state that definitive conclusions cannot be drawn due to the cross-sectional nature of our study, but our results do suggest that OPG is not a reliable marker for insulin resistance.
Author contributions

Design and conduct of the study (EPO’S, DTA, AA, CJT, DJO’G, DS), data collection and analysis (EPO’S, DTA, CD, LP, GK, ND, RC), data interpretation (EPO’S, DTA, CD, PO’S, DJO’G, DS), manuscript writing (EPO’S, DTA, CD, DJO’G, DS).

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

REFERENCES