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Brief Reports

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

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ABSTRACT

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/m^2, $P<.001$) 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 α 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 °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