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Osteopontin and osteoprotegerin levels in type 2 diabetes and their association with cardiovascular autonomic function

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ABSTRACT

Aims: Osteopontin (OPN) and osteoprotegerin (OPG) are bone metabolism biomarkers potentially associated with nerve function. We evaluated the association of cardiovascular autonomic nerve function, OPN, and OPG in 50 individuals with type 2 diabetes mellitus (T2DM).
Methods: RR-variation during deep breathing (i.e., mean circular resultant (MCR) and expiration/inspiration (E/I) ratio) was used to assess parasympathetic nerve function. Participants' demographics, HbA1c, 25-hydroxyvitamin D (25(OH)D), BMI, HOMA-IR, calcium, parathyroid hormone, creatinine, OPN, and OPG were determined.
Results: Using stepwise multiple linear regression analysis with MCR or E/I ratio as the dependent variable, OPN was independently associated with reduced autonomic function. A previous report showed a significant association of cardiovascular autonomic function with age, 25(OH)D insufficiency, and the interaction of age × 25(OH)D insufficiency. Here we report a novel association for OPN and its interaction with age indicating that for those who are younger, elevated OPN levels are related to a greater loss of autonomic function (MCR model $R^2 = 0.598$, $p < 0.001$; E/I model $R^2 = 0.594$, $p < 0.001$).
Conclusion: Our results suggest that OPN is associated with reduced parasympathetic function, particularly in younger individuals with T2DM. Further studies are needed to determine if OPN is neuroprotective, involved in the pathogenesis of autonomic dysfunction, or a bystander.

1. Introduction

Osteopontin (OPN) and osteoprotegerin (OPG), two bone metabolism biomarkers, are key elements in vascular remodeling and have been shown to be elevated in various disease states (e.g., hypertension) (Stepien et al., 2011; Tousoulis et al., 2013). OPN is a multifunctional protein with pleiotropic physiological functions. It is expressed in multiple human cell types (e.g., endothelial cells, vascular smooth muscle cells) and in cells of the nervous system in rodents (Jander et al., 2002; Stepien et al., 2011; Wright et al., 2014). OPN plays a role in both acute and chronic inflammation (Mazzali et al., 2002). Elevated plasma levels of OPN have been reported in individuals with various neurodegenerative diseases (Brown, 2012). OPG is a secretory glycoprotein and a member of the tumor necrosis factor alpha receptor family (Guzel et al., 2013). OPG production occurs in many tissues including bone, endothelial cells and vascular smooth muscle cells. Normally, it circulates in the blood at lower levels than in the tissues (Guzel et al., 2013) but elevated circulating levels of OPG have been reported in individuals with type 2 diabetes mellitus (T2DM), particularly in the presence of microvascular complications (Knudsen et al., 2003).

Diabetes is a leading cause of neuropathy with sensorimotor and autonomic neuropathies being the two main types. Persons with cardiovascular autonomic nerve dysfunction may suffer from orthostatic hypotension, exercise intolerance, intraoperative instability, silent myocardial ischemia, and increased risk of mortality (Maser & Lenhard, 2003; Vinik, Erbas, & Casellini, 2013). The etiology of diabetic neuropathy is multifactorial with metabolic, neurovascular, and inflammatory components. Three studies in persons with diabetes have demonstrated an association between higher OPG levels and peripheral neuropathy (Nybo, Poulsen, Grauslund, Henriksen, &

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Rasmussen, 2010; Tavintharan et al., 2014; Terekeci et al., 2009). OPN, in rodents, has been shown to be upregulated in denervated motor pathways with increased OPN protein expression in denervated Schwann cells following nerve injury (Wright et al., 2014). Schwann cell expression of OPN regulated by axon-derived signals has been demonstrated in sural nerve biopsies in humans (Jander et al., 2002). Given that there is a paucity of studies in humans that have explored an association with cardiovascular autonomic neuropathy and glycoproteins involved in bone homeostasis, the objective in this study was to determine if there is an association between cardiovascular autonomic nerve fiber function, OPG, and OPN.

2. Subjects and methods

2.1. Subjects

Individuals with T2DM (n = 50) were evaluated at the Diabetes and Metabolic Research Center, Christiana Care Health System, Newark, DE, USA. Participants in this study cohort have been previously described (Maser, Lenhard, Sneider, & Pohlig, 2015; Maser, Lenhard, & Pohlig, 2015). Here we report data pertaining to the association among OPN, OPG, and autonomic function. In brief, participants were eligible for the study if they were ≥18 years old with T2DM. Individuals were excluded if they had: (1) a history of known cardiac problems (e.g., myocardial infarction, acute myocardial ischemia, percutaneous coronary interventions, etc.); (2) changes (e.g., change in dose) in vitamin D, antidiabetes and antihypertensive medications 2 months before participation in the study; and (3) stage 3b or greater chronic kidney disease. This study had approval of the Institutional Review Board of Christiana Care Corporation and each participant gave written informed consent before participating in the study.

2.2. Cardiovascular autonomic function tests

Autonomic function was performed following an overnight fast. Participants were asked not to take any prescribed or nonprescription medications, not to consume tobacco products, caffeine-containing and alcoholic beverages, and not to engage in any vigorous exercise 8–10 h prior to testing. Cardiovascular autonomic function (i.e., RR-variation during deep breathing) was measured using the ANS2000 ECG Monitor and Respiration Pacer (DE Hokanson, Inc., Bellevue, WA, USA), as previously described (Maser & Lenhard, 2003). In this study, RR-variation during deep breathing performed for six minutes was measured by vector analysis (i.e., mean circular resultant [MCR]) and by the expiration/inspiration (E/I) ratio of the first six breath cycles. The MCR is resistant to effects of ectopic beats, whereas the E/I ratio is affected by ectopic beats (Schumer, Joyner, & Pfeifer, 1998). In this study cohort, the E/I results for six participants were labeled as missing.

2.3. Blood analytes

Specific enzyme-linked immunosorbent assays (ELISA) were used for the measurement of OPG (ALPCO, Salem, NH, USA) and OPN (R&D Systems, Minneapolis, MN, USA). Samples for OPG, OPN, leptin, and adiponectin were analyzed at the Nemours Biomedical Research & Analysis Laboratory, Jacksonville, FL, USA. The measurement of other analytes (i.e., 25-hydroxyvitamin D [25(OH)D], insulin, C-peptide, glucose, parathyroid hormone, calcium, serum creatinine, leptin, adiponectin, and HbA1c) and the method for calculating homeostasis model assessment for insulin resistance (HOMA-IR) have been previously reported in detail (Maser, Lenhard, Sneider, et al., 2015; Maser, Lenhard, & Pohlig, 2015).

2.4. Statistical analysis

Descriptive data are reported as mean ± SD and for those variables non-normally distributed the median ± semi-interquartile ranges are also provided. Pearson and Spearman rank correlation coefficients were used to evaluate potential bivariate associations between bone metabolism biomarkers (i.e., OPN, OPG), demographics (e.g., gender, BMI), metabolic parameters (e.g., HbA1c, HOMA-IR, parathyroid hormone, calcium, serum creatinine, leptin, total adiponectin, 25(OH)D insufficiency (i.e., <75 nmol/L)), and measures of cardiovascular autonomic function. Stepwise linear regression procedures, where the dependent variables were MCR or E/I ratio, were used to assess for potential independent associations of OPN, OPG, demographic and metabolic parameters. Normality was tested and if violated, a natural logarithmic transformation or nonparametric test was used.

3. Results

Table 1 provides participants' clinical characteristics. Oral antidiabetes agents were used by 48% of the study cohort, 12% used insulin/injectable agents, while 40% used both. Twenty-six percent had peripheral neuropathy determined via physician diagnosed signs and symptoms, while 14% were taking medications used to treat neuropathy.

Significant Pearson correlations for OPG levels included leptin ($r = 0.30$, $p = .037$), calcium ($r = 0.33$, $p = .021$), gender ($r = 0.40$, $p = .004$), and MCR ($r = -0.30$, $p = .036$). Significant Spearman rank correlations for OPN levels included systolic blood pressure ($r = 0.33$, $p = .018$), calcium ($r = 0.31$, $p = .027$), and OPG ($r = 0.29$, $p = .039$).

Stepwise linear regression selection procedures (Table 2) were performed regressing the MCR or E/I ratio on potential independent variables which included age, gender, BMI, HOMA-IR, HbA1c, leptin, total adiponectin, parathyroid hormone, calcium, serum creatinine, 25(OH)D insufficiency, OPG, and OPN. We had previously reported that age, 25(OH)D insufficiency, and their interaction were significantly associated with both measures of nerve function (Maser, Lenhard, & Pohlig, 2015). The stepwise procedure returned a final model of not only age, 25(OH)D insufficiency, and their interaction but also included OPN as a significant variable. OPG, however, was not selected as a significant covariate. Since it was previously shown that age interacted with 25(OH)D insufficiency, the interaction of age with OPN was also included in the model. It should be noted that the age by OPN interaction was significant for the E/I model and was borderline

Table 1
Clinical characteristics of the participants (n = 50).

Variables	Mean ± SD	Median ± SIQR[a]
Age (years)	63 ± 10	–
Duration (years)	13 ± 8	13 ± 5
Male/female (n)	21/29	–
Systolic blood pressure (mmHg)	125 ± 13	–
Diastolic blood pressure (mmHg)	73 ± 7	–
HbA1c (%)	7.3 ± 1.2	7.0 ± 0.7
HbA1c (mmol/mol)	56 ± 13	53 ± 8
Body mass index (kg/m^2)	32.7 ± 5.3	–
HOMA-IR	2.1 ± 1.2	1.8 ± 1.0
Leptin (μg/L)	22.9 ± 17.2	15.7 ± 11.9
Total adiponectin (mg/L)	6.9 ± 5.4	5.9 ± 2.1
25-hydroxyvitamin D (nmol/L)	75 ± 27	–
Parathyroid hormone (ng/L)	36 ± 16	–
Calcium (mmol/L)	2.4 ± 0.1	–
Serum creatinine (μmol/L)	75.7 ± 22.3	70.7 ± 13.3
Osteoprotegerin (pmol/L)	5.0 ± 2.0	–
Osteopontin (ng/mL)	64.5 ± 21.7	56.8 ± 12.4

HOMA-IR: homeostasis model assessment for insulin resistance.
[a] Median and Semi-Interquartile Ranges (SIQR) are provided for variables that were non-normally distributed.