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Insulin resistance is associated with Fibroblast Growth Factor-23 in stage 3–5 chronic kidney disease patients☆,☆☆

Jocelyn S. Garland [a,b,*], Rachel M. Holden [a,b], Robert Ross [c], Michael A. Adams [d], Robert L. Nolan [b,e], Wilma M. Hopman [b,f], A. Ross Morton [a,b]

[a] Department of Medicine, Queen's University, Kingston, ON, Canada
[b] Queen's University Vascular Calcification Investigators, Queen's University, Kingston, ON, Canada
[c] School of Kinesiology and Health Studies, Departments of Endocrinology and Metabolism, Queen's University, Kingston, ON, Canada
[d] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada
[e] Department of Radiology, Queen's University, Kingston, ON, Canada
[f] Clinical Research Center, Kingston General Hospital, and Department of Community Health and Epidemiology, Queen's University, Kingston, Ontario, Canada

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ABSTRACT

Aim: To determine the associations between insulin resistance, fibroblast growth factor 23 (FGF-23), and coronary artery calcification (CAC) in chronic kidney disease (CKD) patients.
Introduction: FGF-23 is associated with atherosclerosis and cardiovascular disease, but its association with insulin resistance in CKD has not been explored.
Subjects: Cross sectional study of 72 stage 3–5 CKD patients receiving care in Ontario, Canada.
Materials and Methods: Insulin resistance was measured by the homeostasis model assessment of insulin resistance (HOMA-IR), FGF-23 was measured by carboxyl terminal enzyme linked immunoassay (ctFGF-23) and CAC was measured by multi-slice computed tomography.
Results: Median HOMA-IR was 2.19 μU/ml (interquartile range 1.19 to 3.94). Patients with HOMA-IR > 2.2 had greater ctFGF-23 (179.7 vs 109.6; $P = 0.03$), and 40% higher log CAC scores (2.09 ± 0.87 vs 1.58 ± 1.26; $P = 0.049$). Multivariable linear regression adjusted for 1,25 dihydroxyvitamin D, kidney function, and parathyroid hormone revealed insulin resistance was a risk factor for greater log ctFGF-23 levels (log HOMA IR $\beta = 0.37$; 95% confidence interval 0.14 to 0.59; $P = 0.002$).
Conclusions: Insulin resistant CKD patients demonstrated higher FGF-23 levels, and increased CAC, while PO_4 levels remained normal, suggesting a potential link between insulin resistance and PO_4 homeostasis in CKD.

1. Introduction

Micro-puncture studies have identified the transporter for renal proximal tubular phosphorus re-absorption, which is coupled with sodium (Greger, Lang, Marchand, & Knox, 1997): the apical type 2 sodium-dependent phosphorus co-transporter (NaPi-II). The NaPi-II co-transporter has various subtypes, but in humans, the NaPi-IIa is mainly responsible for phosphorus re-absorption (Prié & Friedlander, 2010). Maintenance of phosphorus homeostasis is mediated, in part, by the action of counter-regulatory hormones which act to influence renal phosphorus re-absorption. Parathyroid hormone (PTH) decreases the abundance of NaPi-II co-transporters, decreasing phosphorus re-absorption (Zhao & Tenenhouse, 2000). In contrast 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) increases NaPi-II co-transporter abundance (Katai et al., 1999), increasing renal phosphorus re-absorption. Fibroblast growth factor 23 (FGF-23) is a hormone secreted by osteoctyes and is one of the most important regulators of phosphorus homeostasis (Jüppner, Wolf, & Salusky, 2010). When secreted, FGF-23 acts to reduce renal phosphorus re-absorption, (thereby increasing renal phosphorus excretion), by reducing the expression of the kidney proximal tubule NaPi-II co-transporters (Shimada et al., 2004). In CKD patients, as nephron mass decreases, renal phosphorus elimination becomes impaired, and thus FGF-23 increases in an effort to maintain normo-phosphatemia (Gutierrez et al., 2005; Gutierrez et al., 2009). Consequently, it has been suggested

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* Corresponding author. Room 2043 Etherington Hall, Queen's University, Kingston, Ontario, Canada, K7L 3N6. Tel.: +1 613 533 3207; fax.: +1 613 533 2306.
E-mail address: garlandj@queensu.ca (J.S. Garland).

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that increased FGF-23, in CKD, is an early biomarker indicating renal phosphorus homeostasis is disrupted, even in the absence of hyperphosphatemia (Gutierrez et al., 2005; Gutierrez et al., 2009; Jüppner et al., 2010).

An action of insulin that has not been widely studied is its direct involvement in renal phosphorus handling. Insulin also directly induces the NaPi-II, and is anti-phosphaturic, promoting increased renal phosphorus re-absorption (Murer, Hernando, Forster, & Biber, 2000). Insulin resistance is a condition characterized by an impaired physiological response of peripheral tissues to the metabolic effects of insulin action (Reaven, 2004), which occurs, in part, because of decreased insulin receptor expression in tissues that are involved in energy homeostasis (eg. liver and skeletal muscle)(Catena et al., 2003). The insulin receptor is expressed in the kidney, and ^{125}I-labeled insulin has the greatest binding (per length renal tubule) in the kidney proximal convoluted tubule (Hammerman, Rogers, Hansen, & Gavin, 1984). In the kidney, unlike skeletal muscle or hepatic tissue, the state of insulin resistance does not appear to involve down-regulation of insulin receptors. On the contrary, an important observation was made by Sechi et al. when they demonstrated that, despite insulin resistance, insulin receptor number remains preserved in the kidney (Sechi et al., 1996).

The relationship between insulin resistance and FGF-23 has not been investigated previously; however, Wahl et al. have observed that FGF-23 levels are greater in CKD patients who have diabetes (Wahl et al., 2012). Since insulin directly induces the NaPi-II, and is anti-phosphaturic, we sought to evaluate the impact of insulin resistance on FGF-23 levels in 72 pre-dialysis stage 3–5 CKD patients, while accounting for other hormones known to impact on renal NaPi-II expression and FGF-23: parathyroid hormone (PTH), and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). Our primary objective was to determine the association between insulin resistance and FGF-23 in stage 3–5 pre-dialysis CKD patients (not receiving insulin therapy). Our secondary objective was to explore the relationship between insulin resistance and coronary artery calcification in Stage 3–5 pre-dialysis CKD patients.

2. Materials and methods

In 2005, 174 pre-dialysis CKD patients were enrolled in a study of coronary artery calcification (CAC) in CKD (Holden et al., 2010). The full methods are described elsewhere (Holden et al., 2010), but patients were eligible to participate if they were greater than 18 years of age and had stage 3–5 CKD (not requiring dialysis and excluding acute kidney injury and insulin treated diabetes mellitus). All patients who had CAC scores measured in 2005 were invited to undergo repeat multi-slice CT (MSCT) scan for quantification of CAC in 2009, and had repeat clinical and biochemical assessments performed. Of the original 174 patients, 17 patients died, 31 patients progressed to dialysis, 5 were transplanted, 7 were discharged to the care of their family physician, 2 had moved, and 5 were lost to follow-up. This left 107 patients, and 95 agreed to participate. Of these, one CAC score was not interpretable due to motion artefact, and 22 patients were receiving insulin therapy and were excluded (as plasma insulin levels were being measured to determine HOMA-IR), leaving 72 patients who had data for CAC, insulin resistance, and FGF-23. All patients gave informed consent, and the study protocol was approved by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

National Kidney Foundation criteria were applied to diagnose CKD (National Kidney Foundation, 2004). Diagnoses of hypertension were documented as per 2006 Canadian Hypertension Education Program Guidelines (Hemmelgarn et al., 2006), and diabetes mellitus as per the Canadian Diabetes Association criteria (Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2003). Metabolic syndrome was defined as fulfilling at least 3 of 5 criteria from the National Cholesterol Education Program Adult Treatment Panel III 2005 criteria (Grundy et al., 2005).

2.1. Laboratory Measures

Fasting blood samples taken in 2009 were analyzed at Kingston General Hospital's Core Laboratory, including serum creatinine (Jaffe rate method, Beckman Coulter UniCel DxC 800 SYNCHRON Clinical System assay, traceable to isotope dilution mass spectroscopy), glucose, phosphorus, total calcium, intact parathyroid hormone (iPTH),(chemoluminescent immunoassay, Beckman Coulter UniCel DxI 800 Access Immunoassay System, Beckman Coulter Inc., Fullerton CA), albumin, high-sensitivity C-reactive protein (hsCRP),(Beckman Coulter UniCel DxC 600/800 SYNCHRON Clinical System, Beckman Coulter Inc., Fullerton CA), total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and triglycerides.

Blood samples were stored at −80 °C, and after a single freeze–thaw cycle the following were measured from plasma in duplicate at the Ontario Cancer Biomarker Network, Toronto, ON, Canada: 1,25-dihydroxyvitamin D, enzyme immunoassay (Immunodiagnostic Systems Inc., Fountain Hills, Arizona), 25-hydroxyvitamin D, enzyme immunoassay (Immunodiagnostic Systems Inc., Fountain Hills, Arizona), insulin (single measure) Human Serum Adipokine (Panel B) Kit Protocol Immunoassay, (Milliplex Analytes, Millipore Corp, St. Charles, MI), and plasma carboxyl terminal FGF-23, (ctFGF-23) (measured in duplicate), enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH).

The 4-variable MDRD Study equation (Levey et al., 1999), re-expressed for standardized creatinine (Levey et al., 2006), was used to estimate kidney function (estimated glomerular filtration rate (eGFR)). Albuminuria was detected by the urinary albumin-to-creatinine ratio (UACR). Weight and height data were collected on each individual to calculate body mass index (BMI) in kg/m^2. Abdominal obesity was defined as a waist circumference of >88 cm in women and >102 cm in men (Grundy et al., 2005). Insulin resistance was assessed using the following validated formula: homeostasis model assessment of insulin resistance (HOMA-IR) = (fasting glucose [mmol/l] × fasting insulin [μU/ml])/22.5 (Ascaso et al., 2003).

2.2. Coronary Artery Calcification Measurement:

CAC scores were evaluated using the General Electric (GE) VCT 64 slice helical CT scanner, (Waukesha, Wisconsin, USA) and data were processed by Smartscore software, version 3.5 from GE Medical Systems (Waukesha, Wisconsin, USA). The GE VCT 64 slice helical CT scanner scans and reconstructs 8 slices simultaneously, using a step and shoot technique. Each slice is 2.5 mm in thickness with no overlap. Images were acquired with prospective gating technique using a discrete algorithm (Wexler et al., 1996). The total CAC score was generated as per the Agatston method and reported in Agatston units (AU) (Agatston, Janowitz, & Hildner, 1990).

2.3. Statistical methods

Statistical differences between the levels of HOMA-IR were analyzed using the Student's t test, Chi square test or the Mann–Whitney test, as appropriate. Data are expressed as means and standard deviations, medians and inter-quartile ranges (IQR), or percentages, as appropriate. Statistical differences in FGF-23 among three groups were analyzed using the one-way ANOVA test. Bivariate analysis was performed to evaluate the associations between log FGF-23, insulin resistance (HOMA-IR) and other a priori chosen risk factors, including factors important in phosphorus homeostasis (iPTH, phosphorus, 25-hydroxyvitamin D, and 1,25-Dihydroxyvitamin D),

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