Visceral fat and coronary artery calcification in patients with chronic kidney disease

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ABSTRACT

Background. Abdominal fat is a metabolically active tissue which has been associated with cardiovascular events and death in chronic kidney disease (CKD) patients. We explore here the association between surrogates of abdominal fat and coronary artery calcium score (CACS).

Methods. Cross-sectional analysis of 232 non-dialysis-dependent CKD patients Stages 3–5 (median age 60 [25th–75th percentile 52–67] years; 60% men). Visceral adipose tissue (VAT) and CACS were assessed by computed tomography. Surrogates of abdominal fat included VAT and waist circumference (WC).

Results. VAT was positively associated with CACS in univariate analysis (ρ = 0.23). Across increasing VAT quartiles, patients were older, more often men and smokers. Although increasing VAT quartiles associated with higher glomerular filtration rate and leptin, better nutritional status (subjective global assessment) as well as larger muscle stores and strength, they were also more insulin resistant (HOMA-IR), dyslipidemic and inflamed (C-reactive protein and white blood cells). In addition, CACS were incrementally higher. Clinically evident coronary artery calcification (CACS ≥ 10 Agatston) was present in 63% of the patients. Both increased visceral fat (odd ratio 1.60 [95% CI 1.23–2.09] per standard deviation increase) and increased WC (1.05 [1.01–1.12] per cm increase), augmented the odds to present calcification. Such associations remained statistically significant after extensive multivariate adjustment for confounders.

Conclusions. Abdominal fat is associated with coronary artery calcification in non-dialysis dependent CKD patients, supporting its potential role as a cardiovascular risk factor in uremia.

INTRODUCTION

Obesity is a well-known risk factor for cardiovascular disease (CVD); abdominal obesity, and especially visceral adipose tissue (VAT), may play a causal role in this association by acting as an active endocrine organ [1]. In the community, abdominal obesity is more strongly linked to the presence and incidence of hypertension, diabetes, chronic kidney disease (CKD), metabolic syndrome, CVD and mortality, than metrics of overall adiposity (e.g. body mass index, BMI) [2–8].

CKD patients are subjected to accelerated atherosclerosis and frequently suffer from vascular calcification, especially
Visceral fat and coronary calcium score in CKD

**Materials and Methods**

**Patients and study design**

This is an observational study with ongoing patient recruitment aimed to evaluate the association between traditional, novel and uremic risk factors with cardiovascular and general morbidity in CKD patients with disease Stages 3–5 prior to initiation of dialysis. The Malnutrition, Inflammation and Vascular Calcification (MIVC) cohort started in March 2010 and includes consecutive adult patients recruited at the outpatient clinic of the Hypertension and Nephrology Division at Dante Pazzanese Institute of Cardiology in São Paulo, Brazil. The presence of CKD was confirmed by glomerular filtration rate (GFR) based on 24-h urinary creatinine clearance (GFR < 60 mL/min/1.73 m²) and, in older patients, also by the 24-h urinary albumin excretion ≥30 mg/24 h. Exclusion criteria were age below 18 years and above 80 years, clinical signs of acute infection during the month preceding the inclusion, active cancer or liver disease at the time of evaluation, previous diagnosis of immunological diseases and unwillingness to participate in the study. Up to September 2012, there were 278 patients included in this cohort. For the purpose of the present study, we excluded patients who did not undergo CT assessment (n = 15) and those with previous surgical coronary revascularization (n = 31). Thus, we here studied 232 non-dialysis-dependent CKD Stages 3–5 patients (median age 60 [25th–75th percentile 52–67] years; 60% men). The local Ethics Committee approved the study and informed consent was obtained from each patient.

**Anthropometric parameters and nutritional status evaluation**

Anthropometric parameters, including body weight, height, body mass index (BMI; body weight divided by squared height), WC (measured at midway between the lowest lateral border of the ribs and the uppermost lateral iliac crest) and skinfold thicknesses (assessed by a conventional Harpenden® Skinfold Caliper) were evaluated at the recruitment. Lean body mass was estimated by skinfold thickness according to Durnin et al. [26], and lean body mass index (LBMI) was calculated according to Kyle et al. [27] and expressed as kg/m².

The 7-point scale subjective global assessment (SGA) questionnaire [28] was used to evaluate nutritional status. For the purposes of the study, malnutrition was defined as an SGA score <6. Handgrip strength was evaluated on the dominant hand, using a manual dynamometer (Baseline® Hydraulic Hand Dynamometer, NexGen Ergonomics, Inc., Canada). Before using the dynamometer, patients were familiarized with the device. Subjects stood with both arms extended sideways from the body with the dynamometer facing away from the body and were instructed to grip the dynamometer with maximum strength in response to a voice command. The measurements were repeated three times, and the highest value was noted for the study.

**Laboratory parameters**

Morning blood samples were taken after an overnight fast for generation of plasma and serum which were stored at −70°C, if not analyzed immediately. Serum high-sensitivity C-reactive protein (CRP) was measured by immune-turbidimetry, plasma 25-hydroxy-vitamin D by high-performance liquid chromatography [29] and serum leptin by a commercial ELISA kit (LDN, Nordhorn, Germany). GFR was estimated by creatinine clearance from 24-h urinary samples, and 24-hour urinary albumin excretion was analyzed by turbidimetry. White blood cell count and circulating levels of glucose, total cholesterol, triglycerides, albumin, serum intact parathyroid hormone (PTH), phosphorus, ionized calcium and insulin were analyzed using certified methods at the Department of Laboratory Medicine at Dante Pazzanese Institute of Cardiology. Insulin resistance was calculated by the homeostasis model assessment [HOMA-IR: fasting serum insulin (µU/mL) * fasting plasma glucose (mmol/L)/22.5] [30].

**Blood pressure assessment**

Blood pressure (BP) measurements were performed in the upper arm with digital sphygmomanometer (HEM-705CP; Omron Healthcare®). The measurements were repeated three times, according to the recommendations of the JNC7 report [31], and the mean of the two last measurements was recorded.

**CT imaging procedure**

Thoracic and abdominal scan imaging were performed by a 64-slice CT scanner (Toshiba CT scanner Aquillion 64, Toshiba Medical Systems, Japan) at the moment of recruitment. All subjects were examined in the supine position with both arms stretched above the head. The CT data was transferred to a remote workstation (Vitrea 2, version 4.0.0.0, Vital Images, Plymouth, Minnesota) for post-processing and subsequent evaluation.
Coronary artery calcium scoring

Data were acquired with a collimation of $64 \times 0.5$ mm and a tube rotation time of 400 ms, and tube current of 300 mA at 120 kV. Coronary calcium score was determined by a single radiologist blinded to the clinical and biochemical aspects of the patient, using dedicated software (Vitrea 2), based on the Agatston method [32], where coronary calcification was identified as a lesion with an area $> 1 \text{ mm}^2$ and a peak intensity $> 130 \text{ Hounsfield Units (HU)}$; the score is obtained by multiplying the area of each calcified lesion by a weighing factor corresponding to the peak pixel intensity for each lesion. A score $\geq 10$ Agatston was selected to define positive CAC because it has a high interscan variability into the interval from 0 to 10 [33]. For the present analysis, we used the total calcium score (i.e. summing scores of each coronary artery). Patients with coronary artery stents ($n = 19$), did not undergo this examination.

Visceral and subcutaneous fat

Measurements of visceral and subcutaneous fat were made by means of a cross-sectional abdominal CT at the umbilicus level, with a slice thickness of 2 mm. Total adipose tissue areas were then calculated by delineating the abdomen with an electronic graph pen and computing the adipose tissue surface by using an attenuation range of $-150$ to $-50 \text{ HU}$. Visceral fat was distinguished from subcutaneous fat by tracing along the fascial plane defining the internal abdominal wall. The area of each compartment was measured in cm$^2$. Total adipose tissue per abdomen with an electronic graph pen and computing the adipose tissue surface by using an attenuation range of $-150$ to $-50 \text{ HU}$. Visceral fat was distinguished from subcutaneous fat by tracing along the fascial plane defining the internal abdominal wall. The area of each compartment was measured in cm$^2$. Total adipose tissue area was calculated as the sum of the visceral and subcutaneous fat areas. The same skilled radiographer performed all measurements.

Statistical analyses

The variables were expressed as mean $\pm$ SD, median (inter-quartile range, IQR) or as absolute ($n$) and relative ($\%$) values, as appropriate. Correlations between fat tissues depots and coronary artery calcium score (CACs) with selected variables were evaluated by Spearman’s rank correlation test ($\rho$). Differences between groups were compared by Kruskal–Wallis test or $\chi^2$ analysis, as appropriate. Crude and adjusted logistic regression analyses were performed to evaluate the association between fat depots and WC with the presence of CAC (depicted as a CACs $\geq 10$ Agatston). Statistical significance was set at the level of $P < 0.05$, and the statistical analyses were performed using the SPSS software version 13.0 (SPSS, Inc., Chicago, IL, USA) and SAS version 9.3 (SAS Campus Drive, Cary, NC, USA).

RESULTS

Clinical characteristics

There were 110 patients (47%) with diabetes mellitus (DM) and 56 patients (24%) with previous diagnosis of ischemic heart disease; 136 patients (59%) were former or current smokers and 63 patients (27%) were diagnosed with malnutrition. The main CKD etiology was DM (90 patients; 39%), followed by hypertensive nephrosclerosis (58 patients; 25%), unknown etiologies (44 patients; 19%), chronic glomerulonephritis (17 patients; 7%) and other (23 patients; 10%). Average BMI was $29.3 \pm 6.1 \text{ kg/m}^2$ and 137 individuals were considered obese (BMI $\geq 30 \text{ kg/m}^2$) at inclusion. A total of 213 patients underwent CAC assessment, and among these, 134 patients (63%) showed clinical signs of vascular calcification (CACs $\geq 10$ Agatston).

Univariate associates of VAT and CACs

In univariate analysis, VAT was positively correlated with age ($\rho = 0.16$; $P = 0.01$), BMI ($\rho = 0.64$; $P < 0.001$), WC ($\rho = 0.79$; $P < 0.001$), LBMI ($\rho = 0.56$; $P < 0.001$), handgrip strength ($\rho = 0.36$; $P < 0.001$), GFR ($\rho = 0.21$; $P = 0.01$), triglycerides ($\rho = 0.35$; $P < 0.001$), white blood cell count ($\rho = 0.29$; $P < 0.001$), CRP ($\rho = 0.21$; $P = 0.001$), leptin ($\rho = 0.39$; $P < 0.001$) and CACs ($\rho = 0.23$; $P = 0.001$). VAT negatively correlated with total cholesterol ($\rho = -0.13$; $P = 0.043$) and high-density lipoprotein (HDL) cholesterol ($\rho = -0.33$; $P < 0.001$). In non-diabetics ($n = 122$), HOMA-IR positively correlated with VAT ($\rho = 0.41$; $P < 0.001$). CAC was positively correlated with age ($\rho = 0.57$; $P < 0.001$) and white blood cell count ($\rho = 0.19$; $P = 0.004$), whereas it correlated negatively with total cholesterol ($\rho = -0.18$; $P = 0.009$) and PTH ($\rho = -0.17$; $P = 0.010$). CACs did not associate with BMI.

Patient characteristics according to quartiles of visceral fat

Table 1 describes general patient characteristics according to quartiles of visceral fat distribution with the two middle quartiles combined. Across increasing quartiles, patients were older, more often smokers and men. They also had a higher GFR. Regarding body composition parameters, patients had a higher BMI, a larger WC, more subcutaneous fat and more muscle stores (LBMI and handgrip strength) as the visceral fat categories increased. The percentage of malnourished patients was gradually smaller. Whereas HOMA-IR (in non-diabetics), triglycerides, white blood cell count and CRP concentration were increased, HDL cholesterol was incrementally reduced across increasing VAT quartiles. Both CACs and leptin values were significantly increased across the VAT groups considered (Figure 1).

Association between visceral fat and WC with CAC

To explore the independent association between abdominal fat and the presence of CAC, Table 2 shows a series of logistic regression models with gradual multivariate adjustment with CACs ($\geq 10$ Agatston) as the dependent variable, and with visceral fat or WC as the independent variables. In crude analysis, every standard deviation increase in visceral fat increased the odds to present calcification by 60% (OR 1.60 [95% CI 1.23–2.09]). These associations remained significant after gradual adjustment for potential confounders, including surrogates of altered bone metabolism (model 2), malnutrition and inflammation (model 3). Adjustment for leptin made these associations slightly stronger (model 4). Similarly, every centimeter increase in WC increased the odds to present calcification by 5% (1.05 [1.01–1.12]). This association did not
<table>
<thead>
<tr>
<th>Variables</th>
<th>Visceral fat groups</th>
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<tbody>
<tr>
<td></td>
<td>Low quartile (n = 58)</td>
</tr>
<tr>
<td>Visceral fat (cm²)</td>
<td>74 (32–93)</td>
</tr>
<tr>
<td>Clinical and demographical data</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 (48–65)</td>
</tr>
<tr>
<td>Men (n, %)</td>
<td>33 (57%)</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>20 (35%)</td>
</tr>
<tr>
<td>Ischemic heart disease (n, %)</td>
<td>13 (22%)</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>37 (64%)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>152 (131–175)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>82 (70–97)</td>
</tr>
<tr>
<td>Glomerular filtration rate (mL/min/1.73 m²)</td>
<td>14.9 (11.9–24.1)</td>
</tr>
<tr>
<td>Anthropometric and nutritional parameters</td>
<td></td>
</tr>
<tr>
<td>Malnutrition (n, %)</td>
<td>29 (50%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 ± 4.2</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81 (77–90)</td>
</tr>
<tr>
<td>Subcutaneous fat (cm²)</td>
<td>144 (75–197)</td>
</tr>
<tr>
<td>Lean body mass index (kg/m²)</td>
<td>18.4 ± 2.4</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>26 (22–36)</td>
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<tr>
<td>Metabolic and inflammatory markers</td>
<td></td>
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<tr>
<td>HOMA-IR</td>
<td>1.04 (0.63–2.35)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>184 (159–222)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>47 (39–61)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>117 (88–150)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7 (3.4–4.1)</td>
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<tr>
<td>Urinary albumin excretion (mg)</td>
<td>557 (147–2079)</td>
</tr>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.16 (1.05–1.22)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.3 (3.8–5.0)</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>266 (125–409)</td>
</tr>
<tr>
<td>25-OH vitamin D (ng/mL)</td>
<td>44.9 (32.1–66.4)</td>
</tr>
<tr>
<td>White blood cell count (unit/mm³)</td>
<td>6200 (5200–7125)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.4 (0.9–7.9)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>5.5 (3.2–23)</td>
</tr>
<tr>
<td>Vascular calcification</td>
<td></td>
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<tr>
<td>Coronary Artery Calcium Score (Agatston)</td>
<td>5 (00–138)</td>
</tr>
</tbody>
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Continued
change in magnitude with gradual multivariate adjustment for confounders. Subcutaneous fat did not associate with CAC neither in crude nor in adjusted models (data not shown).

DISCUSSION

Clinically evident coronary calcification was present in 63% of the screened patients in our study. This high prevalence agrees with previous studies in similar patient cohorts [11–13] and shows the commonness of this condition in CKD. At the same time, 59% of our patients were considered obese, underlining the epidemics of obesity in the CKD population [14]. The present study shows for the first time in non-dialysis CKD patients that both conditions may be cross-sectionally linked, as accumulation of visceral fat in the abdominal region was independently associated with CAC in this relatively large sample of non-dialysis-dependent patients with CKD Stages 3–5. We confirm this observation by using two surrogates of abdominal fat: WC and the gold-standard computer-tomography quantification of VAT.

Although the role of overall adiposity (e.g. high BMI) as a risk factor in CKD seems to differ before and after dialysis initiation [34], there is no disagreement on the direct association reported between abdominal fat and worse patient outcomes along the whole CKD spectrum [14, 16, 35–37]. Overall adiposity may represent a source of energy and wealth to stand the consequences of malnutrition/wasting, and in our study, patients with increased VAT were indeed less often malnourished and with larger both adipose and muscle stores. However, excess abdominal adiposity may be, at the same time, a direct cardiovascular risk factor by virtue of the endocrine activity of both adipocytes and adipose tissue macrophages [38]. In CKD patients, abdominal fat has been associated with inflammation, insulin resistance, dyslipidemia and oxidative stress [7, 37, 39–41]. Likewise, our study also showed increased systemic inflammation, increased insulin resistance and altered blood lipids in patients with augmented VAT. It has been suggested that the uremic milieu up-regulates the expression of pro-inflammatory and oxidative stress genes in adipocytes [42]. Inflammation is per se a cause of insulin resistance [43], but excessive release of free fatty acids from lipolysis into the portal vein may also contribute [44]. In vitro studies suggest that visceral adipocytes are more lipolytically active than subcutaneous [45, 46].

The main finding of our study is the novel association between VAT, WC and the presence of coronary calcification in non-dialysis-dependent CKD patients. These results are in agreement with previous reports in community-based studies [4, 6, 18–20, 47], altogether adding further support to the
Table 2. Crude and adjusted logistic regression analysis predicting for the presence of coronary artery calcification with either visceral fat or waist circumference in 213 non-dialysis-dependent CKD Stages 3–5 patients

<table>
<thead>
<tr>
<th>Odd ratios (95% CI) for the presence of coronary artery calcification</th>
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<tr>
<td>Visceral fat, per SD</td>
</tr>
<tr>
<td>Model 1</td>
</tr>
<tr>
<td>Model 2</td>
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<tr>
<td>Model 3</td>
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<td>Model 4</td>
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</table>

Patients with a coronary arteries calcium score <10 Agatston were taken as reference (n = 79). SD, standard deviation.

'Model 1’ included the adjustment for age, sex, systolic blood pressure, body mass index, smoking, comorbidities (diabetes mellitus and ischemic heart disease), HDL-cholesterol and glomerular filtration rate.

'Model 2' includes the variables of Model 1 plus markers of mineral metabolism (PTH, phosphorus, ionized calcium and 25-OH vitamin D).

'Model 3’ includes the variables of Model 2 plus CRP, and presence of malnutrition (according to SGA).

'Model 4’ includes the variables of Model 3 plus leptin.

*Pseudo $R^2 = 0.26$

*Pseudo $R^2 = 0.24$.

concept of abdominal fat being a risk factor for CVD. At the same time, our findings expand those of a previous report showing that VAT was associated with carotid calcification in 77 patients undergoing hemodialysis [48]. Similar to non-renal patients with CVD [49], BMI was not associated with CACs in our study, and the association between abdominal fat and CACs was independent of overall adiposity. Our data underlines the importance of assessing regional fat distribution rather than overall adiposity [17, 36, 37]. A strength of the current study is the demonstration of this association by two independent surrogates of abdominal fat. Not surprisingly, the association between WC and CACs was not as robust [50], as WC is a very rough measure that includes both VAT and subcutaneous adipose tissue.

The underlying mechanisms of vascular calcification in CKD are not fully characterized, but may possibly involve disturbances at the level of PTH and calcium/phosphate metabolism, inflammatory cytokines, as well as proteins related to vascular calcification and bone turnover [51]. Possibly due to this multifactorial genesis, the association between abdominal fat and CACs in our study was not too strong. However, our analyses suggest that such association is independent of many of those potentially ‘calcifying’ risk factors. There is, indeed, mounting evidence to suggest that the links between abdominal adipose tissue and calcification may be causally connected. The experimental observations of adiponectin [52] and leptin [53, 54] taking part in the regulation of bone turnover suggest the existence of a reciprocal communication via endocrine signaling between fat and bone [55]. Accordingly, the bone-derived protein osteocalcin stimulates gene expression in beta cells and adipocytes [56], while other bone remodeling factors, such as osteopontin [57] or fetuin-A [58], are expressed in fat tissue in a direct proportion to the degree of obesity. It is of interest to bring into focus other potential mechanisms of leptin, such as disruption of endothelial function through inhibition of endothelial nitric oxide synthase [59]. Leptin has indeed been reported to be an independent predictor of CACs in non-diabetic individuals [25]. As expected, leptin concentration in our study was increased across augmented VAT categories and also associated with CACs. Adjusting for leptin (thus within the causal pathway) only slightly strengthened the association between VAT and CACs. The cross-sectional nature of our analysis and the impact of uremic retention on circulating leptin levels may obscure these relationships and makes the interpretation of our findings difficult.

To conclude, this observational study reports an independent direct association between abdominal fat tissue and CAC in non-dialysis-dependent patients with CKD Stages 3–5. Although causality is impossible to infer from observational studies, our finding supports the concept of abdominal fat as a cardiovascular risk factor also in CKD patients. It should be interpreted as hypothesis generating and may hopefully serve as a starting point for further mechanistic studies on the specific contribution of VAT to the vascular calcification/ossification process in uremia. If so, an interesting aspect that warrants further study is the potential of excess visceral fat to accelerate vascular calcification in uremia. Such a hypothesis is plausible, given recent observations linking the metabolically active epicardial fat accumulation with 3-year CAC progression in asymptomatic diabetic patients without prior history of CVD [60].

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CONFLICT OF INTEREST STATEMENT
None declared.

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