Nephrol Dial Transplant (2013) 28 (Suppl. 4): iv152–iv159
doi: 10.1093/ndt/gft250
Advance Access publication 5 July 2013

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

Antonio Carlos Cordeiro[1,2],
Abdul Rashid Qureshi[2],
Bengt Lindholm[2],
Fernanda Cassullo Amparo[3],
Antonio Tito-Paladino-Filho[4],
Marcela Perini[4],
Fernanda Silvestre Lourenço[4],
Ibraim Masciarelli Francisco Pinto[4],
Celso Amodeo[1]
and Juan Jesús Carrero[2]

[1]Department of Hypertension and Nephrology, Dante Pazzanese Institute of Cardiology, São Paulo, Brazil,
[2]Divisions of Renal Medicine and Baxter Novum, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden,
[3]Department of Nutrition, Dante Pazzanese Institute of Cardiology, São Paulo, Brazil and
[4]Department of Radiology, Dante Pazzanese Institute of Cardiology, São Paulo, Brazil

Correspondence and offprint requests to:
Antonio C. Cordeiro; E-mail: accordeirojr@uol.com.br

Keywords: adiposity, atherosclerosis, central obesity, malnutrition, wasting

ORIGINAL ARTICLE

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 ($\rho = 0.23$). 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 $\geq$ 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

coronary artery calcification (CAC) [9, 10]. Clinically evident CAC is already present at early stages of renal disease [11, 12] and heralds a poor prognosis [13]. In non-dialysis-dependent CKD, overweight, obesity and abdominal obesity are also increasingly common [14]. Measures of central obesity in these patients have been associated with important cardiovascular risk factors [15] and have been shown to strongly predict both cardiovascular events and mortality [16, 17].

Emerging evidence at the community level describes intriguing links between abdominal obesity measures and CAC [18–23], which have been in part attributed to VAT's endocrine activity [24, 25]. In this study, we hypothesized that abdominal obesity is associated to CAC in non-dialysis-dependent CKD patients, establishing links between regional adiposity and CVD risk in renal disease. Abdominal obesity was assessed both by simple waist circumference (WC) and by the gold standard assessment of computed tomography (CT), allowing the quantification of visceral and subcutaneous abdominal tissue stores.

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 morbi-mortality 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 Sao 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 m^2) 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/m^2.

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 × 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 mm^2 and a peak intensity >130 Hounsfield Units (HU); the score is obtained by multiplying the area of each calcified lesion by a weighting factor corresponding to the peak pixel intensity for each lesion. A score ≥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 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 abdominal 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 ± SD, median (interquartile 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 (ρ). Differences between groups were compared by Kruskal–Wallis test or χ^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 ≥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 ± 6.1 kg/m^2 and 137 individuals were considered obese (BMI ≥ 30 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 ≥ 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 ($\rho = 0.21$; $P = 0.001$), 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 (≥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 1. Clinical and laboratory data in 232 non-dialysis-dependent CKD Stages 3–5 patients grouped according to increasing quartiles of computer tomography-estimated visceral fat[a,b]

Variables	Visceral fat groups			
	Low quartile ($n = 58$)	Middle quartiles ($n = 116$)	High quartile ($n = 58$)	P-value
Visceral fat (cm^2)	74 (32–93)	155 (134–179)	261 (224–302)	–
Clinical and demographical data				
Age (years)	55 (48–65)	62 (54–69)	61 (54–69)	0.003
Men (*n*, %)	33 (57%)	62 (53%)	45 (78%)	0.007
Diabetes (*n*, %)	20 (35%)	62 (53%)	28 (48%)	0.061
Ischemic heart disease (*n*, %)	13 (22%)	28 (24%)	15 (26%)	0.910
Smoking (*n*, %)	37 (64%)	56 (48%)	42 (72%)	0.006
Systolic BP (mmHg)	152 (131–175)	149 (131–170)	145 (135–161)	0.827
Diastolic BP (mmHg)	82 (70–97)	77 (70–89)	79 (74–86)	0.352
Glomerular filtration rate ($mL/min/1.73\ m^2$)	14.9 (11.9–24.1)	21.6 (13.4–33.7)	23.5 (11.8–35.7)	0.018
Anthropometric and nutritional parameters				
Malnutrition (*n*,%)[b]	29 (50%)	24 (21%)	10 (17%)	<0.001
BMI (kg/m^2)	24.4 ± 4.2	29.4 ± 5.4	33.8 ± 5.3	<0.001
Waist circumference (cm)	81 (77–90)	96 (90–105)	107 (101–114)	<0.001
Subcutaneous fat (cm^2)	144 (75–197)	230(158–293)	274 (202–353)	<0.001
Lean body mass index (kg/m^2)	18.4 ± 2.4	19.9 ± 3.0	22.6 ± 3.1	<0.001
Handgrip strength (kg)	26 (22–36)	33 (27–42)	39 (30–47)	<0.001
Metabolic and inflammatory markers				
HOMA-IR[c]	1.04 (0.63–2.35)	1.87 (1.05–3.08)	1.97 (1.29–3.78)	0.002
Total cholesterol (mg/dL)	184 (159–222)	180 (148–235)	166 (144–211)	0.177
HDL cholesterol (mg/dL)	47 (39–61)	42 (35–51)	38 (32–43)	<0.001
Triglycerides (mg/dL)	117 (88–150)	156 (117–207)	178 (141–236)	<0.001
Albumin (g/dL)	3.7 (3.4–4.1)	3.9 (3.5–4.2)	3.9 (3.6–4.2)	0.672
Urinary albumin excretion (mg)	557 (147– 2079)	768 (121–2104)	1424 (268–3097)	0.164
Ionized calcium (mmol/L)	1.16 (1.05–1.22)	1.14 (1.09–1.21)	1.13 (1.05–1.20)	0.400
Phosphorus (mg/dL)	4.3 (3.8–5.0)	4.1 (3.4–4.9)	4.2 (3.7–4.8)	0.816
PTH (pg/mL)	266 (125–409)	157 (98–293)	176 (101–379)	0.076
25-OH vitamin D (ng/mL)	44.9 (32.1–66.4)	46.4 (28.6–63.3)	45.5 (24.4–66.7)	0.690
White blood cell count ($unit/mm^3$)	6200 (5200–7125)	7150 (6043–8600)	7885 (6525–9475)	<0.001
CRP (mg/L)	2.4 (0.9–7.9)	3.5 (1.5–6.8)	6.0 (1.5–11.9)	0.020
Leptin (ng/mL)	5.5 (3.2–23)	20.5 (10.3–43.2)	26.9 (13.1–61.5)	< 0.001
Vascular calcification				
Coronary Artery Calcium Score (Agatston)[d]	5 (00–138)	71 (00–330)	198 (13–850)	< 0.001

Continued

Table 1. Continued

Variables	Visceral fat groups			
	Low quartile (n = 58)	Middle quartiles (n = 116)	High quartile (n = 58)	P-value
Coronary Artery Calcium Score groups (n, %)[d]				
<10 Agatston	30 (56%)	37 (35%)	12 (22%)	
10–399 Agatston	18 (33%)	47 (45%)	24 (45%)	0.003
≥400 Agatston	6 (11%)	21 (20%)	18 (33%)	

Data are presented as median (25th–75th percentiles), mean ± standard deviation or absolute (n) plus relative (%) values. Patient grouping was done by quartiles of VAT distribution, lumping together the middle quartiles.
[b]Malnutrition was assessed by subjective global assessment <6.
[c]HOMA-IR was reported in non-diabetics only. Number of non-diabetic patients across tertiles: 38/54/30.
[d]Coronary artery calcium score was available in 213 patients.

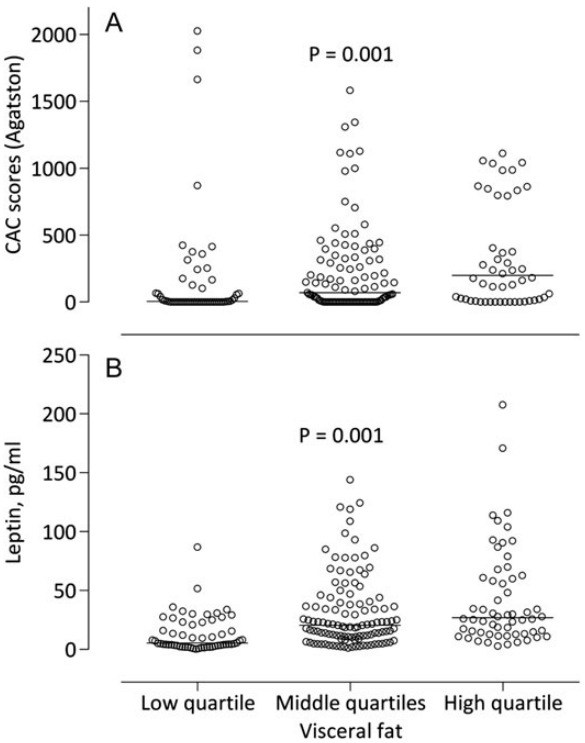

FIGURE 1: Coronary Artery Calcium (CAC) scores (Agatston, n = 213, **A**) and serum leptin concentration (n = 232, **B**) across increasing quartiles of computer tomography-assessed visceral fat in non-dialysis-dependent patients with CKD Stages 3–5.

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

Odd ratios (95% CI) for the presence of coronary artery calcification			
Visceral fat, per SD	1.60 (1.23–2.09)	Waist circumference, per cm	1.05 (1.01–1.12)
Model 1	1.90 (1.25–2.91)	Model 1	1.06 (0.99–1.12)
Model 2	1.87 (1.21–2.86)	Model 2	1.06 (1.00– 1.12)
Model 3	1.97 (1.26–3.09)	Model 3	1.06 (1.00–1.13)
Model 4	2.01 (1.29–3.18)[a]	Model 4	1.06 (1.01–1.13)[b]

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.
[a]Pseudo $R^2 = 0.26$
[b]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].

ACKNOWLEDGEMENTS

We thank the patients and personnel involved in the creation of this cohort. This study is supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo [FAPESP]

(Process number: 2010/16593-2) and Adib Jatene's Foundation. J.J.C. acknowledges grant support from the Swedish Research Council and the Loo and Hans Osterman's Foundation. Baxter Novum is the result of a grant to the Karolinska Institutet from Baxter Healthcare Corporation. Baxter Healthcare Corporation employs B.L.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol 2004; 15: 2792–2800
2. Goodpaster BH, Krishnaswami S, Resnick H *et al.* Association between regional adipose tissue distribution and both type 2 diabetes and impaired glucose tolerance in elderly men and women. Diabetes Care 2003; 26: 372–379
3. Hayashi T, Boyko EJ, Leonetti DL *et al.* Visceral adiposity is an independent predictor of incident hypertension in Japanese Americans. Ann Intern Med 2004; 140: 992–1000
4. Goodpaster BH, Krishnaswami S, Harris TB *et al.* Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch Intern Med 2005; 165: 777–783
5. Yusuf S, Hawken S, Ounpuu S *et al.* Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet 2005; 366: 1640–1649
6. Fox CS, Massaro JM, Hoffmann U *et al.* Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007; 116: 39–48
7. Carrero JJ, Cordeiro AC, Lindholm B *et al.* The emerging pleiotrophic role of adipokines in the uremic phenotype. Curr Opin Nephrol Hypertens 2010; 19: 37–42
8. Czernichow S, Kengne AP, Stamatakis E *et al.* Body mass index, waist circumference and waist-hip ratio: which is the better discriminator of cardiovascular disease mortality risk?: evidence from an individual-participant meta-analysis of 82 864 participants from nine cohort studies. Obes Rev 2011; 12: 680–687
9. Stenvinkel P, Carrero JJ, Axelsson J *et al.* Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? Clin J Am Soc Nephrol 2008; 3: 505–521
10. Foley RN, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol 1998; 9(12 Suppl): S16–S23
11. Tomiyama C, Higa A, Dalboni MA *et al.* The impact of traditional and non-traditional risk factors on coronary calcification in pre-dialysis patients. Nephrol Dial Transplant 2006; 21: 2464–2471
12. Porter CJ, Stavroulopoulos A, Roe SD *et al.* Detection of coronary and peripheral artery calcification in patients with chronic kidney disease stages 3 and 4, with and without diabetes. Nephrol Dial Transplant 2007; 22: 3208–3213
13. Watanabe R, Lemos MM, Manfredi SR *et al.* Impact of cardiovascular calcification in nondialyzed patients after 24 months of follow-up. Clin J Am Soc Nephrol 2010; 5: 189–194
14. Kramer HJ, Saranathan A, Luke A *et al.* Increasing body mass index and obesity in the incident ESRD population. J Am Soc Nephrol 2006; 17: 1453–1459
15. Evans PD, McIntyre NJ, Fluck RJ *et al.* Anthropomorphic measurements that include central fat distribution are more closely related with key risk factors than BMI in CKD stage 3. PLoS One 2012; 7: e34699
16. Elsayed EF, Tighiouart H, Weiner DE *et al.* Waist-to-hip ratio and body mass index as risk factors for cardiovascular events in CKD. Am J Kidney Dis 2008; 52: 49–57
17. Kramer H, Shoham D, McClure LA *et al.* Association of waist circumference and body mass index with all-cause mortality in CKD: the REGARDS (Reasons for Geographic and Racial Differences in Stroke) Study. Am J Kidney Dis 2011; 58: 177–185
18. Ho JS, Cannaday JJ, Barlow CE *et al.* Comparative relation of general, central, and visceral adiposity measures for coronary artery calcium in subjects without previous coronary events. Am J Cardiol 2009; 104: 943–946
19. Ohashi N, Yamamoto H, Horiguchi J *et al.* Visceral fat accumulation as a predictor of coronary artery calcium as assessed by multislice computed tomography in Japanese patients. Atherosclerosis 2009; 202: 192–199
20. Choi SY, Kim D, Oh BH *et al.* General and abdominal obesity and abdominal visceral fat accumulation associated with coronary artery calcification in Korean men. Atherosclerosis 2010; 213: 273–278
21. Ohashi N, Yamamoto H, Horiguchi J *et al.* Association between visceral adipose tissue area and coronary plaque morphology assessed by CT angiography. JACC Cardiovasc Imaging 2010; 3: 908–917
22. Jensky NE, Criqui MH, Wright CM *et al.* The association between abdominal body composition and vascular calcification. Obesity (Silver Spring) 2011; 19: 2418–2424
23. Imai A, Komatsu S, Ohara T *et al.* Visceral abdominal fat accumulation predicts the progression of noncalcified coronary plaque. Atherosclerosis 2012; 222: 524–529
24. Maahs DM, Ogden LG, Kinney GL *et al.* Low plasma adiponectin levels predict progression of coronary artery calcification. Circulation 2005; 111: 747–753
25. Qasim A, Mehta NN, Tadesse MG *et al.* Adipokines, insulin resistance, and coronary artery calcification. J Am Coll Cardiol 2008; 52: 231–236
26. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974; 32: 77–97
27. Kyle UG, Schutz Y, Dupertuis YM *et al.* Body composition interpretation. Contributions of the fat-free mass index and the body fat mass index. Nutrition 2003; 19: 597–604
28. Adequacy of dialysis and nutrition in continuous peritoneal dialysis: association with clinical outcomes. Canada-USA (CANUSA) Peritoneal Dialysis Study Group. J Am Soc Nephrol 1996; 7: 198–207
29. Kobold U. Approaches to measurement of vitamin D concentrations—mass spectrometry. Scand J Clin Lab Invest Suppl 2012; 243: 54–59

30. Matthews DR, Hosker JP, Rudenski AS *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419
31. Chobanian AV, Bakris GL, Black HR *et al.* Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003; 42: 1206–1252
32. Agatston AS, Janowitz WR, Hildner FJ *et al.* Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990; 15: 827–832
33. Jain T, Peshock R, McGuire DK *et al.* African Americans and Caucasians have a similar prevalence of coronary calcium in the Dallas Heart Study. J Am Coll Cardiol 2004; 44: 1011–1017
34. Zoccali C, Seck SM, Mallamaci F. Obesity and the epidemiology and prevention of kidney disease: waist circumference versus body mass index. Am J Kidney Dis 2011; 58: 157–159
35. Kamimura MA, Carrero JJ, Canziani ME *et al.* Visceral obesity assessed by computed tomography predicts cardiovascular events in chronic kidney disease patients. Nutr Metab Cardiovasc Dis 2012 Jul 25. [Epub ahead of print]. PMID:22841184
36. Postorino M, Marino C, Tripepi G *et al.* Abdominal obesity and all-cause and cardiovascular mortality in end-stage renal disease. J Am Coll Cardiol 2009; 53: 1265–1272
37. Cordeiro AC, Qureshi AR, Stenvinkel P *et al.* Abdominal fat deposition is associated with increased inflammation, protein-energy wasting and worse outcome in patients undergoing haemodialysis. Nephrol Dial Transplant 2010; 25: 562–568
38. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006; 444: 875–880
39. Gohda T, Gotoh H, Tanimoto M *et al.* Relationship between abdominal fat accumulation and insulin resistance in hemodialysis patients. Hypertens Res 2008; 31: 83–88
40. Odamaki M, Furuya R, Ohkawa S *et al.* Altered abdominal fat distribution and its association with the serum lipid profile in non-diabetic haemodialysis patients. Nephrol Dial Transplant 1999; 14: 2427–2432
41. Ramos LF, Shintani A, Ikizler TA *et al.* Oxidative stress and inflammation are associated with adiposity in moderate to severe CKD. J Am Soc Nephrol 2008; 19: 593–599
42. Witasp A, Carrero JJ, Heimburger O *et al.* Increased expression of pro-inflammatory genes in abdominal subcutaneous fat in advanced chronic kidney disease patients. J Intern Med 2011; 269: 410–419
43. Plomgaard P, Bouzakri K, Krogh-Madsen R *et al.* Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. Diabetes 2005; 54: 2939–2945
44. Nielsen S, Guo Z, Johnson CM *et al.* Splanchnic lipolysis in human obesity. J Clin Invest 2004; 113: 1582–1588
45. Rebuffe-Scrive M, Andersson B, Olbe L *et al.* Metabolism of adipose tissue in intraabdominal depots of nonobese men and women. Metabolism 1989; 38: 453–458
46. McConnaughey MM, Sheets KA, Davis J *et al.* Differences in beta-adrenergic receptor densities in omental and subcutaneous adipose tissue from obese African American and Caucasian women. Metabolism 2004; 53: 247–251
47. Pou KM, Massaro JM, Hoffmann U *et al.* Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 2007; 116: 1234–1241
48. Yamauchi T, Kuno T, Takada H *et al.* The impact of visceral fat on multiple risk factors and carotid atherosclerosis in chronic haemodialysis patients. Nephrol Dial Transplant 2003; 18: 1842–1847
49. Marques MD, Santos RD, Parga JR *et al.* Relation between visceral fat and coronary artery disease evaluated by multidetector computed tomography. Atherosclerosis 2010; 209: 481–486
50. Velludo CM, Kamimura MA, Sanches FM *et al.* Prospective evaluation of waist circumference and visceral adipose tissue in patients with chronic kidney disease. Am J Nephrol 2010; 31: 104–109
51. Cannata-Andia JB, Rodriguez-Garcia M, Carrillo-Lopez N *et al.* Vascular calcifications: pathogenesis, management, and impact on clinical outcomes. J Am Soc Nephrol 2006; 17(12 Suppl 3): S267–S273
52. Shinoda Y, Yamaguchi M, Ogata N *et al.* Regulation of bone formation by adiponectin through autocrine/paracrine and endocrine pathways. J Cell Biochem 2006; 99: 196–208
53. Ducy P, Amling M, Takeda S *et al.* Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell 2000; 100: 197–207
54. Gordeladze JO, Drevon CA, Syversen U *et al.* Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. J Cell Biochem 2002; 85: 825–836
55. Lee NK, Sowa H, Hinoi E *et al.* Endocrine regulation of energy metabolism by the skeleton. Cell 2007; 130: 456–469
56. Ferron M, Hinoi E, Karsenty G *et al.* Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci USA 2008; 105: 5266–5270
57. Gomez-Ambrosi J, Catalan V, Ramirez B *et al.* Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab 2007; 92: 3719–3727
58. Witasp A, Carrero JJ, Hammarqvist F *et al.* Expression of osteoprotegerin in human fat tissue; implications for chronic kidney disease. Eur J Clin Invest 2011; 41: 498–506
59. Cheng KH, Chu CS, Lee KT *et al.* Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. Int J Obes (Lond) 2008; 32: 268–274
60. Yerramasu A, Dey D, Venuraju S *et al.* Increased volume of epicardial fat is an independent risk factor for accelerated progression of sub-clinical coronary atherosclerosis. Atherosclerosis 2012; 220: 223–230

Received for publication: 20.10.2012; Accepted in revised form: 19.4.2013