Annual variation in body fat is associated with systemic inflammation in chronic kidney disease patients Stages 3 and 4: a longitudinal study

Laura Kawakami Carvalho¹, Maria Ines Barreto Silva¹,², Barbara da Silva Vale¹, Rachel Bregman¹,³, Renata Brum Martucci², Juan Jesus Carrero⁴ and Carla Maria Avesani¹,²

¹Clinical and Experimental Physiopathology Program, Rio de Janeiro State University, Rio de Janeiro, Brazil, ²Department of Applied Nutrition, Nutrition Institute, Rio de Janeiro State University, Rio de Janeiro, Brazil, ³Nephrology Division, Rio de Janeiro State University, Rio de Janeiro, Brazil and ⁴Division of Renal Medicine, Karolinska Institutet, Stockholm, Sweden

Correspondence and offprint requests to: Carla Maria Avesani; E-mail: carla.avesani@carrenho.com.br

Abstract

Background. In dialysis patients, cross-sectional studies show that total and abdominal body fat associate with inflammatory markers. Whether this is true in earlier disease stages is unknown. We evaluated the cross-sectional and longitudinal (12-month interval) association between body fat markers and C-reactive protein (CRP) in pre-dialysis chronic kidney disease (CKD) patients.

Methods. We studied, over a period of 1 year, clinically stable CKD patients at Stages 3–4 who were under treatment in a single outpatient clinic. Fifty-seven patients were included and 44 concluded the observational period [males: 66%; age: 62.9±13.9 years; body mass index (BMI): 25.5±5.1 kg/m²; estimated glomerular filtration rate (eGFR): 34±12.3 mL/min/1.73m²]. Total body fat (skinfold thicknesses), waist circumference (WC), laboratory measurements (serum creatinine, total cholesterol, albumin, high-sensitivity CRP and leptin) and food intake (24-h food recall) were assessed at baseline and after 12±2 months.

Results. Most patients had anthropometric parameters in the range of overweight/obesity and none had signs of protein-energy wasting. In univariate analysis, changes (delta: end—baseline) in CRP were associated (P<0.05) with changes in BMI (r=0.39) and WC (r=0.33). In multiple regression analysis, these associations remained significant (P<0.05) even after adjusted by potential confounders (sex, diabetes, baseline age and eGFR).

Conclusions. During a follow-up of 12 months, changes in BMI and WC were directly associated with changes in CRP. Our results support the concept that interventions aimed at reducing weight and/or abdominal adiposity in pre-dialysis CKD patients may also translate into reduced systemic inflammation.

Keywords: adiposity; chronic kidney disease; inflammation; overweight

Introduction

Chronic low-grade inflammation is highly prevalent among chronic kidney disease (CKD) patients, especially in those with end-stage renal disease (ESRD). The reasons for this condition are diverse and may include a variety of factors related to uremia per se and to the dialysis procedure that stimulates the inflammatory response by activating the production of pro-inflammatory cytokines by the macrophages [1]. More recently, the excess of adipose tissue has also been listed as another potential cause of low-grade inflammation in CKD patients [2]. Adipose tissue is now recognized as an endocrine organ and a major site for integration of inflammatory and metabolic pathways [3]. Subcutaneous fat and visceral abdominal fat are the main fat deposit sites in the body, which are biologically distinct, but with one common feature—both display pro-inflammatory and insulin resistance-associated profiles in obese subjects [4]. The cause of the increased circulating pro-inflammatory cytokines during obesity is not totally understood, but it seems to be related to a progressive infiltration of macrophages in the adipose tissue as obesity develops [5]. In addition, the recent paper from Witas et al. [6] showed increased pro-inflammatory genes expression in subcutaneous abdominal fat in patients with advanced CKD, providing a biological insight at the cellular level linking obesity with inflammation in CKD patients. This body of literature explains the
positive association found between total and abdominal body fat and inflammatory markers in both the general population [7, 8] and the CKD patients [9–13]. However, in CKD patients, these observations are limited to cross-sectional studies and mostly in ESRD patients [11–13]. Reports addressing the longitudinal association between changes in inflammatory markers and changes in body fat markers are important to motivate a clinical role of strategies aimed at losing body fat and body weight as a way to diminish or ameliorate the overall state of chronic inflammation present in CKD patients [10]. The high prevalence and incidence of obesity in CKD patients [14] and, the observation that obesity is an independent predictor of both incident CKD [15] and progression to ESRD [16] and the association between obesity, dysmetabolism and the excessive cardiovascular disease in ESRD patients [17, 18] makes this a relevant issue.

In non-CKD obese subjects, recent studies suggested that weight loss can reduce inflammatory markers levels [19–21] and diminish cardiovascular risk factors [22]. Frason et al. [19] demonstrated that reduction in body weight, body mass index (BMI) and waist circumference (WC) was positively associated with reduction in C-reactive protein (CRP) production in overweight and obese individuals without CKD followed >11 years. However, to the best of our knowledge, whether a reduction in body weight or body fat associates with reduction in systemic inflammation in CKD patients is not yet known.

Considering these findings that link body fat with the presence of inflammation, we hypothesized that changes in weight and body fat are associated with changes in serum CRP in CKD patients. We evaluated this in a cohort of pre-dialysis CKD patients followed for 12 months.

Materials and methods

Study design and population selection

This is an observational longitudinal study aimed at following, for 1 year, clinically stable CKD patients at Stages 3 and 4, which were under treatment at the CKD multidisciplinary outpatient clinic at Pedro Ernesto University Hospital (Rio de Janeiro State University—Rio de Janeiro, Brazil). Patients were enrolled between January 2008 and September 2009. Only patients >18 years old, with glomerular filtration rate (GFR) between 59 and 15 mL/min/1.73m² and being followed by the renal dietitian from the CKD outpatient clinic for at least 6 months were included in the study. Exclusion criteria comprised presence of active malignancy diseases, human immunodeficiency virus, acute inflammation, use of immunosuppressive drugs and diabetes mellitus with unstable glucose levels. The diabetic patients (n = 3) included in the study were treated by means of lifestyle changes (diet) and were not receiving insulin therapy nor hypoglycemiant agents. All patients were advised to restrict their protein intake to 0.6–0.8 g of protein/kg/day with 25–30 kcal/kg/day.

From 200 patients being followed by the renal dietitian at the CKD outpatient clinic, 57 patients met the inclusion criteria. Of them, 13 patients did not conclude the observational period due to death (n = 1; 7.7%), commencement of renal replacement therapy (n = 2; 15.4%) and lack of interest/discontinuation of protocol (n = 10; 76.9%). The remaining 44 patients attended a second visit after 12 ± 2 months. Figure 1 depicts a flow chart describing the population selection.

Except for the proportion of males, included patients (n = 44) had similar demographic and clinical characteristics from those who dropped-out (n = 13): males: 29 (66%) versus 5 (38.5%), P = 0.008; age: 62.9 ± 13.9 versus 66.8 ± 13.7 years; BMI: 25.5 ± 5.1 versus 26.6 ± 5.1 kg/m²; eGFR: 34 ± 12.3 versus 34.7 ± 9.9 mL/min/1.73m² and included patients versus dropped-out, respectively.

The protocol was approved by the institutional Ethical Human Research Committee of the Pedro Ernesto University Hospital, and all patients gave written consent before entering in the study.

Methods

Data collection. Patients were assessed at the beginning of the study and again after 12 ± 2 months, collecting clinical, anthropometric and nutritional data at both time points, while demographic data only at baseline. All anthropometric measurements and the 24-h food recall were performed by two experienced renal dietitians.

Anthropometric data. Anthropometric parameters performed included body weight, height, arm circumference, skinfold thickness (SKF) of triceps, biceps, subscapular and suprailiac and WC. Body weight was measured to the nearest 0.1 kg in balance–beam scale. Height was measured with patients standing erect with their head in the Frankfort plane and recorded to the nearest 0.5 cm. BMI was calculated as body weight (kilograms)/height² (meters). Standard ideal body weight was calculated as the patient’s actual body weight multiplied by 100 and divided by the desirable body weight. Desirable body weight was obtained from the Metropolitan Life Insurance tables [23]. SKF was measured on the left arm or in the arm contrary to the arteriovenous fistula, by using a Lange skinfold caliper (Cambridge Instrument, Cambridge, MA) according to standard techniques. WC was measured at the umbilical level by using a flexible plastic tape with graduated scale to the nearest 0.1 cm. Mid-arm muscle circumference (MAMC, in centimeters) was calculated using the formula: arm circumference – (0.314 × triceps SKF) [24]. Standard MAMC and triceps SKF were obtained using the National Health and Nutrition Examination Survey (NHANES) percentile distribution tables adapted by Frisanche [24]. Because SKF cannot be accurately measured in overweight/obese patients, those with BMI ≥25 kg/m² had their body fat assessed according to the validated equations of Weltman et al. [25, 26], which uses WC, body weight and height in the following equation: in men [25]: body fat (%) = (0.31457 × waist circumference (cm)) − (0.10969 × body weight (kg)) + 10.8336; in women [26]: body fat (%) = (0.11077 × waist circumference (cm)) − (0.17666 × height (cm)) + (0.14354 × body weight (kg)) − 51.0330.

For patients with BMI <25 kg/m², body fat was estimated by the sum of SKF according to Durnin and Womersley [27] and then body fat percentage was calculated by the Siri’s equation [28].

Food intake. Food intake was evaluated by a 24-h food recall. Energy and protein intake were calculated using a computer software developed by the Federal University of São Paulo (NUTWIN; São Paulo—Brazil) that contains the US Department of Agriculture tables [29] as the nutrient database. The energy and nutrient content of Brazilian food items were included in the software’s nutrient database. Energy and protein intake were normalized by the desirable body weight and were used to screen patients with protein-energy wasting (PEW) according to the criteria proposed by the International Society of Renal Nutrition and Metabolism (ISNRM) [30].

Laboratory parameters. Patients underwent blood sampling early in the morning, after overnight fasting. Blood samples were centrifuged at 5000 r.p.m. for 10 min. The specimens were then divided into aliquots and...
stored at −80°C until the analysis of CRP and leptin, which were performed at the end of the study. Routine hospital methods were used for the determination of serum creatinine (kinetic method of Jaffé reaction), cholesterol (enzymatic CHOP-POD—esterase–oxidase) and albumin (green bromocresol method). Serum cholesterol and albumin were used for screening patients with PEW according to the criteria proposed by the ISRN [30]. High-sensitivity CRP was measured by enzyme-linked immunosorbent assay (Immunoperoxidase Assay for Determination of CRP in human: Immunology Consultants Laboratory, Inc., Newberg, OR) based on purified protein and polyclonal anti-CRP antibodies. The analyses were done in duplicates and the assay had a sensitivity of 0.01 mg/L. The cutoff limit used for inflammation was CRP >10.0 mg/mL [1].

Serum leptin levels were measured using a human leptin-specific radioimmunoassay kit (Millipore corporation, Billerica, MA), which measures leptin with an assay sensitivity of 0.01 ng/mL. GFR was estimated by the simplified equation from Modification of Diet in Renal Disease study [31].

The cutoff limit used for inflammation was CRP >10.0 mg/L [1].

**Results**

**Baseline characteristics**

Patients were in their sixties and approximately two-thirds were men. The mean BMI was indicative of overweight (BMI ≥25.0 kg/m²) and the mean eGFR was indicative of CKD Stage 3, with 25 patients (56.8%) at Stage 3 and 19 (43.2%) patients at Stage 4. The most frequent cause of CKD was hypertension (n = 25; 57%), followed by tubule interstitial nephritis (n = 5; 11%), Type 2 diabetes (n = 3; 7%), polycystic kidney disease (n = 3; 7%), focal segmental glomerulosclerosis (n = 3; 7%) and undetermined cause (n = 5; 11%).

**Follow-up characteristics**

Table 1 compares the clinical characteristics and the nutritional parameters at baseline and after 12 months of follow-up (n = 44)*

<table>
<thead>
<tr>
<th>Nutritional parameters</th>
<th>Baseline</th>
<th>12 months</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>67.4 ± 14.0fs 68.0 ± 13.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 ± 5.1</td>
<td>25.7 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>25.9 ± 7.7</td>
<td>26.4 ± 7.4</td>
<td>NS</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>38.1 ± 6.8</td>
<td>38.4 ± 7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Men</td>
<td>89.5 ± 11.1</td>
<td>90.1 ± 12.1</td>
<td>NS</td>
</tr>
<tr>
<td>Women</td>
<td>88.6 ± 12.7</td>
<td>89.2 ± 9.4</td>
<td>NS</td>
</tr>
<tr>
<td>MAMC (%)</td>
<td>85.9 ± 11.4</td>
<td>89.7 ± 13.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>4.4 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>0.009</td>
</tr>
<tr>
<td>Protein intake (g/kg/day)</td>
<td>0.9 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Energy intake (kcal/kg/day)</td>
<td>23.1 ± 8.3</td>
<td>21.1 ± 6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>34.1 ± 12.3</td>
<td>32.4 ± 14.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>2.2 ± 0.8</td>
<td>2.4 ± 1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dL)s10</td>
<td>192.7 ± 39.5</td>
<td>184.6 ± 38.8</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin (ng/mL)f</td>
<td>5.4 (1.2, 27.5)f8ns</td>
<td>6.0 (1.3, 32.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>10.5 ± 6.3</td>
<td>10.3 ± 7.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

a NS, not significant.
bPaired t-test or Wilcoxon test, depending on variables’ distribution.

*Mean ± SD.

Median (minimum; maximum).

s = 42 (baseline and after 12 months).

n = 33 (baseline and after 12 months).
BMI, total body fat and WC did not change during the follow-up. In general, an important proportion of patients had anthropometric characteristics indicative of overweight: 44.7% (\(n=21\)) had BMI \(\geq 25\) kg/m\(^2\). In addition, the majority of men (93%) and all women had body fat \(\geq 15\) and 23% (thresholds for obesity [32]), respectively, and 53.3% of men and 17.2% of women had WC \(\geq 88\) cm and 102 cm (thresholds for abdominal obesity according to NCEP/ATPIII [33]), respectively. None of the patients presented PEW at baseline or in the end of the follow-up, according to the criteria proposed by the ISRNM [30]. Of note, standard MAMC increased significantly during the follow-up. Serum albumin levels decreased but remained within the normal range in both assessments. Additionally, protein and energy intake reduced at the end of follow-up and the protein intake approached the recommended values for these patients (0.6–0.75 g/kg/day) [34]. The parameters indicative of renal function (eGFR and serum creatinine) have significantly changed at the end of the observational period, but eGFR remained in the range of CKD Stage 3. Serum cholesterol and CRP have not changed over the follow-up. The leptin level increased significantly at the end of the study.

**Univariate analysis and multivariate associations between changes in CRP and leptin with body composition measurements**

In univariate analyses, whereas changes in CRP were positively correlated with changes in BMI and WC (Table 2; Figure 2a and b, respectively), changes in leptin were only associated with changes in total body fat (Table 2). Changes in CRP and leptin were not associated with changes in eGFR (\(r = 0.14\), \(n=44\) and \(r = -0.01\), \(n=44\), respectively).

In the multiple regression analysis (Table 3), the models, which were adjusted for age, sex, eGFR and diabetes, showed a significant association between changes in CRP and changes in BMI as well as between changes in CRP and changes in WC. The magnitude of this association was that for each unit increase in BMI (kg/m\(^2\)) and WC (cm), an increase of, respectively, 1.94 and 0.45 mg/L in CRP was observed.

**Discussion**

It is well known that CKD is associated with inflammation, and this metabolic milieu may contribute significantly to the excessive cardiovascular disease seen in these patients [35]. Multiple investigations have shown that obesity is associated with inflammation in CKD patients, but these studies have a cross-sectional design and were mostly performed in ESRD patients during dialysis [9–11, 13]. In this study, we demonstrated that changes in CRP, a surrogate of systemic inflammation, was highly and positively associated with changes in markers of adiposity, such as BMI and WC, in a population of CKD patients at Stages 3 and 4 followed for 12 months. In addition, this association remained significant after adjustment for clinical confounders, such as age, sex, eGFR and diabetes. Our investigation therefore supports the notion that body weight and body fat reduction may contribute to the improvement of systemic inflammatory status in CKD Stages 3–4 patients.

Our population included a majority of patients with anthropometric parameters indicative of overweight and obesity, which is in line with recent reports in pre-dialysis patients at CKD Stages 2–5 [9, 10]. One strength of our study is the use

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**Table 2.** Univariate analysis testing the association between 12-month variation in BMI and WC with changes in CRP and leptin (\(n=44\)).

<table>
<thead>
<tr>
<th></th>
<th>(\Delta \text{ CRP (mg/L)}^b)</th>
<th>(\Delta \text{ Leptin (ng/mL)}^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta \text{ BMI (kg/m}^2)</td>
<td>0.39*</td>
<td>0.24</td>
</tr>
<tr>
<td>(\Delta \text{ Total body fat ()}%</td>
<td>0.28</td>
<td>0.40*</td>
</tr>
<tr>
<td>(\Delta \text{ WC (cm)})</td>
<td>0.33*</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*\(\Delta\) (delta 12 months baseline).

^bPearson’s test (\(n=44\)).

^cSpearman rank test (\(n=33\)).

*\(P \leq 0.05\).
of two indicators of adiposity, one of total body fat and the other of central body fat, which allow us to expand previous cross-sectional analyses [9–13]. Altogether, we speculate that differential body fat distribution impacts on pro-inflammatory cytokine secretion and may contribute to the overall inflammatory status. In our study, when we assessed longitudinally the univariate associations between changes in CRP and in body fat markers, we found that delta CRP was positively associated with delta BMI and with delta total body fat. In addition, delta CRP was positively associated with delta BMI. Similar results have been reported previously in studies comprising non-CKD overweight and obese patients [20, 22], but to the best of our knowledge, ours is the first study reporting this in CKD patients at pre-dialysis stages.

The mechanisms underlying the association between changes in adiposity and in inflammatory markers are likely to be explained by the fact that adipose tissue, especially visceral fat, is an important source of adipocytokine synthesis, including leptin, interleukin-6 and tumor necrosis factor-alfa, which attract macrophages that infiltrate among adipocytes, leading to more release of cytokines [5, 36]. Our longitudinal results therefore raise the question of whether controlled strategies aimed at diminishing body weight and body fat are likely to have a beneficial effect in the overall inflammatory status. In addition, because inflammation is strongly associated with cardiovascular disease and mortality in ESRD patients [35], one can anticipate other potential benefits of losing body weight and body fat in diminishing cardiovascular risk. It is important to highlight that in our study, no patients had signs of PEW, and therefore, our results cannot be extrapolated to wasted patients. Other potential benefits of weight loss for obese patients in early CKD stages were previously discussed in a meta-analysis [37] and included a decrease in BMI, proteinuria and in blood pressure for non-surgical interventions, and besides those, a normalization in GFR for surgical interventions. However, in the aforementioned meta-analyses, the impact of losing body weight on diminishing inflammation was not addressed.

In conclusion, our study shows that during a follow-up of 12 months, changes in BMI and WC were directly associated with changes in CRP levels. Further randomized, controlled and longitudinal investigations assessing the potential benefits of body weight and adiposity loss in overweight/obese pre-ESRD patients can be of high importance in routine clinical practice.

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Conflict of interest statement. None declared.

References
7. Lin CC. The relationship of high sensitivity C-reactive protein to percent body fat mass, body mass index, waist-to-hip ratio, and waist circumference in a Taiwanese population. *BMJ Public Health* 2010; 10: 579–585
8. Faber DR, van der Graaf Y, Westerink J et al. Increased visceral adipose tissue mass is associated with increased C-reactive protein in patients with manifest vascular diseases. *Atherosclerosis* 2010; 212: 274–280

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**Table 3.** Multiple regression models testing the association between 12-month variation in BMI and WC with changes in CRP during 12 months of follow-up (n = 44)

<table>
<thead>
<tr>
<th>Dependent variable: Δ CRP (mg/L)</th>
<th>Coefficient β</th>
<th>Adjusted R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ BMI (kg/m²)</td>
<td>1.94</td>
<td>0.18</td>
<td>0.008</td>
</tr>
<tr>
<td>Baseline age</td>
<td>−0.1</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Men</td>
<td>−2.0</td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>Baseline eGFR</td>
<td>−0.03</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7.3</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ WC (cm)</td>
<td>0.45</td>
<td>0.30</td>
<td>0.02</td>
</tr>
<tr>
<td>Baseline age</td>
<td>−0.2</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Men</td>
<td>−3.5</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Baseline eGFR</td>
<td>0.001</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7.1</td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>

*Δ (delta 12 months baseline).

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