Changes in bone mineral density in patients with non-dialysis dependent chronic kidney disease are associated with body composition.

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Abbreviations: BMD: bone mineral density; BMI: body mass index; CKD: chronic kidney disease; CKD-MBD: CKD-mineral and bone disorder; NDD-CKD: non-dialysis dependent CKD; DXA: dual-energy X-ray absorptiometry; eGFR: estimated glomerular filtration rate; HOMA-IR: homeostasis model assessment of insulin resistance; LCI: load-capacity index; LST: lean soft tissue; ALST: appendicular LST; ALSTI: ALST index; PTH: parathormone.

ABSTRACT

Objective: Chronic kidney disease (CKD) and low bone mineral density (BMD) are highly prevalent and can co-exist. Parameters of mineral metabolism are associated with BMD in CKD, but other contributing factors may contribute. The aim of this study was to assess changes in BMD and its determinants in patients with non-dialysis-dependent CKD (NDD-CKD).

Methods: Body composition and biochemical profiles were assessed in a retrospective hospital-based cohort study of patients with NDD-CKD. BMD, lean soft tissue (LST), appendicular LST (ALST), and percentage fat mass were assessed by dual-energy X-ray absorptiometry (DXA).

ALST index (ALSTI, ALST/height^2) and load-capacity index (LCI, fat mass/LST) were calculated. Low BMD was defined as t-score ≤-1.0.

Results: Mean time between assessments was 2.8±1.3 years, 46 patients were included. A reduction in renal function was observed. Changes in body composition included reductions in ALST (p=0.031), ALSTI (p=0.021) and a trend for BMD (p=0.053); and an increase in percentage fat mass (p=0.044) and LCI (p=0.032). Females had a reduction in BMD (p=0.034), ALST (p=0.026), and ALSTI (p=0.037). Patients with low BMD at baseline had lower LST (p=0.013), ALST (p=0.023), and percentage fat mass (p=0.037) than those with normal BMD. Additionally, reductions in LST (p=0.041), ALST (p=0.006), and ALSTI (p=0.008) were observed in patients who had low BMD at baseline, while no significant changes in body composition were observed in those with normal BMD at baseline. The following body composition parameters at baseline were determinants of BMD status at follow-up: LST (OR:0.899, 95%CI:0.829–0.976, p=0.010), ALST (OR:0.825, 95%CI:0.704–0.967, p=0.017), and ALSTI (OR:0.586, 95%CI:0.354–0.968, p=0.037), independent of fat mass, and LCI.

Conclusions: Detrimental body composition changes were observed without changes in body weight; these were more significant in females. Moreover, this is the first longitudinal study showing a protective effect of LST against BMD loss in patients with NDD-CKD.

Keywords: bone mineral density, chronic kidney disease, body composition, muscle loss, osteoporosis.

INTRODUCTION

Chronic kidney disease (CKD) is a consequence of abnormalities in kidneys' structure and/or function, present for more than 3 months [1]. Loss of renal function can contribute to the development of a process known as CKD-mineral and bone disorder (CKD-MBD), which is characterized by disturbances in bone metabolism and morphology, and decreases in bone mineral density (BMD) [2]. In fact, CKD-MBD can start at the initial states of CKD, although it is exacerbated at the commencement of dialysis [3], ultimately contributing to increased cardiovascular disease risk due to vascular calcification [2]. Most studies of bone loss in CKD focus on patients under dialysis treatment [4-7]. Early treatment, including nutritional intervention, may contribute to adequate nutritional status, reduction of disease progression and delayed onset of dialysis in patients with non-dialysis-dependent CKD (NDD-CKD) [8]. This, in turn, can decrease the risk of comorbidities, such as cardiovascular diseases and MBD [8].

As the deterioration of bone health is often observed in CKD, BMD status should be monitored [2]. Dual-energy X-ray absorptiometry (DXA) is used to assess BMD and a t-score is used to diagnose osteopenia (t-score $\leq$-1.0 and >-2.5) and osteoporosis (t-score $\leq$-2.5) [9]. Most studies have focused on biochemical parameters as determinants of bone status in CKD, such as parathormone (PTH), phosphate, calcium, vitamin D, among others [2,3,5]. However, determinants of bone loss are still undetermined, and few studies have analysed the influence of body composition parameters in bone loss. Previous prospective studies have observed that body mass index (BMI) is protective of BMD (6,7), probably due to mechanic load, as the gravitational force on the weight may induce bone remodeling [10]. Nonetheless, specific contributions of fat versus lean soft tissues (LST) are less understood, and LST may have an additional protective factor as observed in cross-sectional studies [11-14].

The physiology of bone and skeletal muscle tissues is comparable and appears to be related [10,15]. Muscle contraction may contribute to bone strength by increasing mechanic load beyond the weight pressure [10]. Besides the biomechanical stimuli, muscle may have paracrine interaction with bone by producing growth factors that stimulates bone mass and function [15]. Reduction in skeletal muscle mass with aging frequently occurs alongside decreases in BMD [10,15,16]. In a previous study, BMD was associated with muscle parameters (i.e., LST, and muscle strength, and quality) in NDD-CKD [14]. However, to the best of our knowledge, studies evaluating longitudinal interactions between changes in bone and LST, or with other muscle parameters have not yet been conducted. Thus, the aim of this study was to evaluate change in BMD, and to identify body composition and biochemical parameters associated with bone loss in patients with NDD-CKD.

METHODS

Study design and population selection

This was a retrospective follow-up study enrolling clinically stable patients (≥ 18 years) with CKD from stage 3a to 5 (NDD-CKD patients). Patients were followed standard medical and nutritional treatment for at least 6 months (nephrologist and renal dietitian) at a nephrology outpatient clinic. Routine appointments were scheduled for 4 to 6 visits per year, depending on the clinical condition and CKD stage; DXA scan was performed as part of the CKD care of patients at the clinic, according to nephrologist requisition. Patients with available DXA body composition measurements at two timepoints were included (n=46). Study exclusion criteria was active malignant disease, glomerulonephritis under immunosuppressive therapy, history of any organ transplantation, human immunodeficiency virus infection, acute inflammation, chronic

lung disease, liver failure, heart failure (class 3 or 4), apparent edema, undergoing dialysis, using immunosuppressive and corticoid drugs, using vitamin D supplements, and using phosphate binders. The study was approved by the human ethics research committee board of the University Hospital. Informed consent was obtained from patients before each time point assessment. Data collection occurred between 2009 and 2015, with an interval of 2.8±1.3 years (95% CI 2.4 to 3.2 y) between baseline and follow-up. Patients were referred to the outpatient clinic at Department of Nephrology for CKD treatment by a multiprofessional team. Patients attended medical/nutritional appointments 4 to 6 times/year depending on their health status and CKD stage. Nutritional counseling was based on guideline recommendations [17], with 0.6-0.8 g protein/kg of actual or adjusted body weight and 20 to 30 kcal/kg/day. Adjusted body weight calculation is described in the subsequent section. Patients were instructed to follow their usual diet and fast for at least 12 hours before blood sample collection, and to avoid strenuous physical activity 72 hours prior to the body composition assessment. Assessments included demographic, anthropometric, body composition, and clinical/laboratorial data.

Anthropometry and body composition

Anthropometric measures were height (to the nearest 0.5 cm) and body weight (to the nearest 0.1 kg). Measurements were assessed using a balance-beam scale with a stadiometer attached to the platform Filizola® (São Paulo, Brazil). Patients were wearing light clothing, without shoes, with an empty bladder, and were standing with their head on Frankfort plane. Measurements were conducted in triplicate and mean values were used. Height and weight were used to calculate the BMI as kg/m^2. The BMI values were used to identify patients in nutritional status classes as normal (BMI 18.5-24.9 kg/m^2 for adults or 22-27 kg/m^2 for older adults) or excess body weight (i.e., overweight/obesity; BMI ≥25 kg/m^2 for adults or > 27 kg $/m^2$ for older

adults) [18,19]. The body weight of the patients with high or low BMI was adjusted as follow: adjusted body weight= 21.7 (adults) or 24.5 (older adults) x height^2.

Body composition parameters were assessed using a GE Lunar iDXA (GE Healthcare, Madison, WI) and the enCore 2008 version 12.20 software (GE Healthcare), with patients wearing minimal clothing and laying in supine position [20]. As preparation for the scan, patients were instructed to fast for 12 h, refrain from vigorous exercise, and maintain their regular diet as prescribed by the registered dietitian [20]. Parameters assessed included total body BMD, fat mass, total LST, and appendicular lean soft tissue (ALST) as the sum of LST of the limbs. The ALST index (ALSTI) was calculated as ASLT/height^2 [21]. Load-capacity index (LCI) integrates the effects of body adiposity and LST to predict disease risk, and was calculated as fat mass/ total LST [22]. T-score is calculated as the number of standard deviations (SDs) of BMD compared to the mean BMD for a sex and ethnicity-matched young adult healthy population [23]. Total body BMD has been shown to have a high accuracy compared to lumbar (L1–L4) BMD in this population [14].

Definitions: T-score was used to define normal BMD (> -1.0) and low BMD (≤ -1.0, combining osteopenia and osteoporosis). ALST and ALSTI were used as proxy for skeletal muscle mass; thus, low muscle mass was defined according to ALSTI as < 7.0 kg/m^2 for men and < 5.5 kg/m^2 for women [21].

Laboratory parameters

Blood biochemical parameters included: creatinine (mg/dL), urea (mg/dL), uric acid (mg/dL), potassium (mEq/L), phosphorus (mg/dL), calcium (mg/dL), glucose (mg/dL), triglycerides (mg/dL), total cholesterol (mg/dL), high- and low-density lipoprotein (HDL and LDL) cholesterol (mg/dL). These parameters were analysed at the University Hospital Central

Laboratory after the blood sample collection using well standardized methods. Insulin (mUI/mL) was analysed in serum stored in a freezer at -80°C by radioimmunoassay using human-insulin kits (Millipore, Billerica, MA, USA), with a sensitivity of 0.2 μU/mL. The estimated glomerular filtration rate (eGFR, mL/min) was calculated with the creatinine equation recommended by the Chronic Kidney Disease Epidemiology Collaboration [24]. The CKD stages were defined according to eGFR: 45-59 (stage 3a), 30-44 (3b), 15-29 (4), and < 15 (5) mL/min/1.73 m^2 [17]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the fasting plasma glucose and fasting insulin concentrations, as recommended [25]. The following reference ranges were used for selected kidney function and mineral metabolism outcomes: phosphorus 3.5 – 5.5 mg/dL [26], calcium 8.4 – 9.5 mg/dL [26], urea 5 – 20 mg/dL [27], and uric acid < 7.0 mg/dL in males and < 6.0 mg/dL in females [28].

Statistical analysis

Normality of data distribution was assessed by the Shapiro-Wilk W-test. Continuous variables were presented as mean ± SD when normally distributed, and as median and interquartile interval when not normally distributed. Outliers were identified by inspection of boxplots, values >1.5 box-lengths from the edge of the box were excluded from analysis. Paired t-test or Wilcoxon signed rank test were used for comparisons between baseline and follow-up according to data distribution. Independent tests, Student t-test or Mann-Whitney test, were used for comparisons between groups, according to distribution patterns. Absolute changes were calculated as values of follow-up - values of baseline, percentual changes were also calculated. All statistical analysis were conducted in the total sample, divided by sex, and in groups according to BMD status (normal or low) at baseline. Logistic regression analysis was performed to assess risk factors at baseline (as independent continuous variable) to identify variables

explaining low BMD at follow-up (as categorical dependent variable). The following models were explored: 1) unadjusted; 2) adjusted for eGFR and age at baseline, sex, and time between baseline and follow-up; 3) adjusted for variables in model 2 + fat mass percentage; 4) adjusted for variables in model 2 + LCI, and 5) adjusted for variables in model 2 + presence of type 2 diabetes, as it may contribute to muscle loss [29]. All logistic regression models were fitted according to Hosmer-Lemeshow test to avoid multicollinearity. Correlation between biochemical parameters and body composition parameters with BMD and t-score were performed using Pearson's or Spearman's correlation according to data distribution. Partial correlations were also performed and adjusted for eGFR at baseline, sex, age at baseline, and time between baseline and follow-up. Chi-square was used to compare group and sex frequencies. The statistical software package IBM® SPSS® version 28 (International Business Machines Inc., USA) was used, and the sensitivity of the analyses was of 95%, considering $p < 0.05$ as significant.

RESULTS

Participant characteristics and biochemical parameters

A total of 46 NDD-CKD patients had a follow-up visit (n=25 [54.3%] were males). At baseline mean age was 64.4±9.8 years, with no differences between sexes (males: 66.2±9.2 years females: 62.3±10.4, p=0.192), 60.9% presented with excess body weight and no patient had BMI below normal. At baseline, most patients had CKD stages 3b (n=19, 41.3%), followed by stages 4 (n=18, 39.1%), 3a (n=7, 15.2%), 2 and 5 (each with n=1, 2.2%). The most common underlying condition was hypertension (n=22, 47.8%), followed by diabetes mellitus (n=8, 17.4%), and other diseases including chronic glomerulonephritis, tubulointerstitial nephritis, and polycystic kidney disease (n=10, 21.8 %) and unknown etiologies (n=6, 13.0%).

Differences in anthropometry (weight and BMI) and blood biochemical parameters between baseline and follow-up are shown in **Table 1**. No significant differences in weight and BMI were observed. As expected, there was an increase in creatinine concentration (p=0.022) and a decrease in the eGFR ($p < 0.001$), reflecting progression of the CKD. Mean yearly reduction in eGFR was -2.67±2.7 mL/min/year (min -7.9, max +3.6). No other significant changes in blood biochemical parameters were observed. Mean values for phosphorus and calcium were within normal limits [26].

The eGFR percentual change was correlated with percentual change in creatinine (rho= -0.887, $p<0.001$) reflecting inherent CKD progression. This correlation was maintained after adjustment for sex, age, and time between baseline and follow-up (r= -0.773, $p<0.001$). Percentual change in urea concentration was correlated with percentual change in eGFR (rho= -0.363, p=0.035), and percentual change in creatinine (rho=0.431, p=0.011). The later was still significant after adjusting for sex, age, and time between baseline and follow-up (r=0.520, p=0.003).

Changes in body composition parameters

Frequency of patients with low BMD (t-score ≤ -1.0) changed from 41.3% at baseline to 45.6% at follow-up (chi^2 p=0.874). Regarding body composition parameters (**Figure 1** and **Supplementary Table 1**), there was a trend for a decrease in BMD (mean difference: -0.7±0.4%, p=0.053), and nearly 5% decrease in ALST (-4.5±1.9%, p=0.031) and ALSTI (-4.6±2.0%, p=0.021). An increase in fat mass (4.5±2.2%, p=0.044), and in LCI (mean difference: 2.72% [-3.55 – 10.16], p=0.032) were observed.

When patients were divided by sex, females had a lower BMD at baseline (p=0.002) and follow-up (p=0.001) compared to males. A change in BMD was observed in females (-1.2±0.5%,

p=0.034), and more than 5% reduction in LST parameters (ALST: -7.2±2.9%, p=0.026, and ALSTI: -6.8±3.1%, p=0.037). There was a trend for an increase in fat mass in females (7.1±4.0%, p=0.078). In males, there was no significant alterations in BMD (-0.3±0.5%, p=0.493), ALST (-2.3±2.5%, p=0.328), ALSTI (-2.8±2.7%, p=0.219), and fat mass (2.4±2.3%, p=0.348). No significant differences in absolute and percentual changes in body composition parameters between sexes was observed (data not shown).

Comparison and changes in body composition parameters according to BMD classification

Patients were divided according to BMD classification at baseline (normal or low BMD). Frequency of males between normal and low BMD groups was similar (baseline: 55.6% and 52.6%, respectively, chi^2 p=0.917; follow-up 56.0% and 52.4%, respectively, chi^2 p=0.959). Differences in body composition between baseline and follow-up, and between groups are presented in **Table 2**. Patients in the low BMD group had lower total LST (p=0.019) and ALST (p=0.023) at both time points (p=0.008 for both) compared to the normal BMD group. Although ALSTI was similar between low and normal BMD groups at baseline, this parameter was lower in the low BMD group compared to normal BMD group at follow-up (p=0.014). In the low BMD group, reductions in total LST (p=0.041), ALST (p=0.006), and ALSTI (p=0.008) were noted, with no changes in the normal BMD group. Patients in the low BMD group also had lower total fat mass at baseline (fat mass percentage: p=0.037), and a trend to increase fat mass (p=0.074), and LCI (p=0.082) at follow-up.

Potential sources of bias were similar between groups with normal and low BMD, i.e., time between assessments, age, eGFR, eGFR loss between assessments, and presence of

diabetes. No differences between groups were observed regarding time between assessments (2.7±1.3 y in normal BMD, and 2.8±1.4 y in low BMD, p=0.773). Additionally, there were no significant differences in mean age between groups at baseline (normal BMD group =59.8±9.8 y, low BMD group =64.7±4.5 y, p=0.097) and follow-up (normal BMD group=62.6±9.8 y, low BMD group= 67.5±5.3 y, p=0.052). Mean eGFR was similar between these groups (baseline: 33.9±8.8 mL/min in normal BMD and 35.9±11.8 mL/min in low BMD, p=0.572; follow-up: 27.8±10.8 mL/min in normal BMD and 28.0±9.3 mL/min in low BMD, p=0.958). The eGFR loss was of -6.1±7.3 mL/min (-18.5±4.9%) in the normal BMD group, and -7.9±6.3 mL/min (-21.2±4.1%) in the low BMD group (p=0.448). Prevalence of diabetes was not different between low and normal BMD groups (chi^2=0.377, p=0.539).

Body composition parameters based on percentual changes during study period (**Figure 2**), were not significantly different when comparing normal versus low BMD groups at baseline. However, as absolute numbers, the low BMD group presented with a higher decrease in total LST, ALST, and ALSTI, and a higher increase in fat mass and LCI.

Predictors of low bone mineral density

To assess risk factors for low BMD at follow-up, a logistic regression analysis was performed with continuous LST parameters at baseline (**Table 3, Supplementary Table 2**). Total LST had a significant protective effect in the unadjusted model, which was maintained in all adjusted models, and it was independent of eGFR at baseline, time between the baseline and follow-up, and of fat mass, LCI, and presence of diabetes. Similarly, ALST was protective in all models, and it was independent of eGFR at baseline, fat mass, LCI, and presence of diabetes. Moreover, ALSTI was protective in all models, and it was independent of eGFR at baseline, sex,

fat mass, LCI, and presence of diabetes. BMD status at follow-up was influenced by sex when the independent variables were ALST and ALSTI.

Correlations between change in t-score and biochemical parameters were significant for changes in uric acid (r= -0.526, p=0.030), and calcium (r=0.375, p=0.038). However, these correlations were not significant after adjusting for sex, age, eGFR at baseline, and time between baseline and follow-up (r= -0.546, p=0.053, and r=0.243, p=0.222, respectively).

Frequency of patients presenting concomitant low BMD and low muscle mass

Patients were divided into four groups (**Figure 3**) according to BMD (classified by t-score) and muscle mass (classified by ALSTI). At baseline, 30.4% presented with low BMD only, 2.2% of patients presented with low muscle mass only, and 10.9% presented with both conditions. At follow-up, there was an increase of 2.2% of patients with low BMD only, 8.7% increase of patients with low muscle mass, and 2.2% increase of those with both conditions (chi^2 p=0.311).

DISCUSSION

This is the first longitudinal study to explore the relationship between BMD and other body composition parameters related to LST and adiposity in patients with NDD-CKD. Low BMD prevalence was high at baseline (~41%) and increased at follow-up (~46%). There was a tendency of decreased BMD that did not reach statistical significance for the entire group, but sex-differences were observed, with a significant reduction in females but not in males. In addition to changes in BMD, we found significant reductions in muscle mass parameters and a significant increase in fat mass and LCI without alterations in BMI. This indicates there were detrimental body composition changes without change in body weight, further highlighting the

limitation of using a simple anthropometric parameter in this population. Further, LST parameters were associated with changes in BMD with no changes in fat mass and LCI.

Reductions in bone mineral density

The CKD-MBD has been associated with poor outcomes, including higher mortality [1,3], but bone loss in CKD has been less studied than mineral biochemical alterations. Bone loss is increased during hemodialysis compared to NDD-CKD. A study following patients with stage 5 CKD undergoing hemodialysis for 2 years found a reduction of -0.03 g/cm^2 (-3.1%) in total hip BMD and -0.02 g/cm^2 (-1.1%) in spine BMD [5]. Longitudinal studies in NDD-CKD are scarce, but studies in the general older adult population have found lower BMD in people with lower kidney function. A cohort study that followed women for 10 years (starting from their 75th birthday onwards), and found a reduction of -0.01 SD in total body t-score, and identified greater bone loss in those with poor (stages 3a – 5) compared to normal (stages 1 – 2) kidney function [30]. A longitudinal study (3 years) with older adults (≥ 65 years) found changes in BMD ranging from 0.01 to -0.03 g/cm^2 depending on the bone site (femoral neck, lumbar spine, or total hip), and lower eGFR was related to lower femoral neck BMD in men but not in women [31]. This is in line with our study, as we observed a reduction of -0.01 g/cm^2 in total body BMD. It is possible that in the present study, greater bone loss could have been detected in more sensitive sites, such as femoral neck, total hip, lumbar spine, or the forearm [32,33].

Interaction between bone and other body composition parameters

Cross-sectional studies have shown moderate to strong correlations between BMD and LST parameters in CKD, varying between r=0.46 (p=0.043) to r=0.632 (p<0.0001) [11,13,14]. Loss of muscle mass and function have been associated with mortality in the context of CKD [34,35]; however, studies showing longitudinal muscle loss are scarce in this population. In a 16-year

longitudinal study, participants with CKD had greater decline in LST than healthy counterparts, and those with CKD and diabetes had even higher LST reduction [36]. Type 2 diabetes contributes to muscle loss, because insulin is an anabolic hormone; thus, there is a decreased protein synthesis in insulin resistance [29].

In the present study, significant differences in body composition were found when patients were divided according to BMD status at baseline. A cross-sectional study showed that higher fat mass and body weight may have a protective effect on BMD due to weight load [14]. In the present study, differences between groups could not be explained by sex, time between assessments, or eGFR; age might be related with BMD. Our findings indicate that people who had low BMD at baseline had worse body composition phenotype at follow-up. Differences in body composition parameters between BMD groups could be related to differences in physical activity levels, which increases not only muscle mass but also BMD [37]. Although differences were not explained by sex, similar alterations in body composition were observed in females but not in males, which may be explained by differences in sexual hormones [38,39].

We showed that 30.4% of participants had low BMD, 2.2% had low ALSTI, and 10.9% had both conditions at baseline. In a previous cross-sectional study, 33.1% of patients had low BMD, 1.4% had sarcopenia, and 7.0% had osteosarcopenia [14]. Differences in prevalence may be explained by differences between low ALSTI versus sarcopenia, defined in the previous study as combined low ALSTI and low muscle strength. Unfortunately, a limitation of the present study is the lack of muscle strength data to evaluate sarcopenia and osteosarcopenia; thus, we assessed low ALSTI and its co-occurrence with low BMD. At the follow-up, there was also an increase in the prevalence of low ALSTI and low BMD, as expected due to the decrease in BMD and LST parameters observed.

Body weight and the frequency of patients with excess body weight (according to BMI criteria) remained stable across both study time-points and no patients presented with a BMI value below normal. Body weight was used to adjust the diet counseling provided at the outpatient clinic, according to recommended guidelines [17,40]. However, diet adherence was not specifically evaluated; consequently, we did not examine associations between dietary intake and body composition. Although patients did not report any concerns about muscle loss, our results showed a significant relationship between changes in LST and BMD. These findings are important for future studies aiming to understand factors related to changes in the body composition in patients with NDD-CKD.

Biochemical factors

As expected, we showed a decrease in renal function with CKD progression, which may contribute to the observed changes in body composition. The relationship of mineral metabolism parameters (PTH and Vitamin D) with BMD and body composition were not analysed in the present study. Reduced vitamin D is one of the onset factors of hyperparathyroidism and is important for calcium homeostasis [41] which can be associated with risk for vascular calcification and, thus, increases in cardiovascular risk [42]. However, a previous cross-sectional study showed no correlations between BMD and PTH and vitamin D [14].

There were no changes in other biochemical parameters, although some were altered in both timepoints compared to reference values for healthy population. For example uric acid was higher than the reference range [28]. Interestingly, we found positive correlations between uric acid and ALST or ALSTI, and an inverse correlation with BMD. Similar results were found in patients with CKD undergoing peritoneal dialysis [43] and kidney transplant patients [44]. Uric acid may be protective of muscle mass as it may be a marker of better nutritional status and

antioxidant capacity [43,44]. However, uric acid induces vitamin D deficiency [45,46], and its crystallization increases oxidative stress and pro-inflammatory cytokines that hinders bone remodeling [45]. Osteoprotective effects of uric acid have been observed in the general population [45], but have not been reported in CKD [46].

Low BMD was present in 41.3% of patients and mean serum levels of phosphorus and calcium were within normal range throughout the study. Patients in the present study were not on therapy with phosphate binders, antiresorptive medications, and bisphosphonates. Biochemical abnormalities, such as hyperphosphatemia may be less frequent in patients with NDD-CKD as homeostatic mechanisms are activated to promote phosphorus excretion to maintain normal serum phosphorus and calcium levels [1,17,47-49]. The clinical practice guideline for CKD-MBD[1] suggests that patients with biochemical abnormalities of CKD-MBD, low BMD, and/or fragility fractures may receive antiresorptive therapy, such as bisphosphonates[1]. However, definitive trials evaluating antiresorptive therapy in patients with NDD-CKD are lacking[50], some side effects may occur (e.g., acute kidney injury in CKD stages 3a to 5), and there is concern that bisphosphonates would induce low-turnover bone disease[1]. Thus, antiresorptive therapy for osteoporosis in patients with CKD should be individualized, used with caution, and addressing first the underlying renal osteodystrophy[1].

Strengths and limitations

This study includes the longitudinal design which allowed the assessment of long-term (~3 years) changes in body composition in people with NDD-CKD. Furthermore, DXA is a gold-standard technique for BMD assessment, which was also used to assess LST. Future studies could assess skeletal muscle mass, such as computerized tomography, magnetic resonance imaging, ultrasound, and D_3-creatine dilution. Muscle strength can also be assessed (e.g., hand-

grip strength) to determine sarcopenia and osteosarcopenia. We also analysed biochemical parameters related of body composition changes. Other biochemical factors can influence the bone and muscle loss and should be explored in longitudinal studies, including PTH, vitamin D, fibroblast growth factor-23, klotho, inflammatory status, and acidosis. Other confounding factors also deserve to be explored, such as physical activity, which was not controlled for in the present study. We did not assess menopause in the present study, but the mean age of females indicate that most females were post-menopause throughout the study.

CONCLUSION

The change in BMD in this study was partially explained by changes in LST parameters (total LST, ALST, and ALSTI). Patients presented a general worsening of body composition without weight changes, which was more significant in those with low BMD at baseline. LST parameters at baseline were associated with a protective effect against low BMD at follow up, which was independent of factors such as age, renal function, and diabetes. Changes in body composition were only significant in females. Thus, women in our study were at higher risk for osteoporosis and sarcopenia, and specific factors should be considered, such as menopause.

Our findings may contribute to the treatment approach to prevent CKD-MBD in NDD-CKD in clinical practice. Even if patients are not malnourished, it is fundamental to monitor changes in lean and muscle tissues in this population, which can be used as a strategy to prevent bone loss. As such, strategies to maintain muscle mass in this population should be developed, which could be useful to prevent bone loss and its complications.

Practical Application: As body weight did not change during follow-up, but LST decreased along with a trend to decrease BMD, it is crucial to assess body composition in people at risk for low BMD. The results of the present study highlight the need to focus on nutritional therapeutic measures targeting the lean soft tissue to lower the risk of bone mineral disorder in patients with chronic kidney disease.

FIGURES AND TABLES

Table 1. Patients' characteristics and biochemical parameters at baseline and follow-up.

Parameter	Baseline	Follow-up	P*
Weight (kg)	68.8±14.7	69.9±15.9	0.180
BMI (kg/m^2)	26.3±4.4	26.7±5.0	0.180
Creatinine (mg/dL)	2.0 (1.6 – 2.3)	2.3 (1.8 – 3.1)	**0.022**
eGFR (mL/min)	33.8 (27.5 – 41.0)	25.5 (19.6 – 38.3)	**<0.001**
Urea (mg/dL)	59.5 (51.8 – 80.0)	76.5 (52.8 – 92.3)	0.156
Uric acid (mg/dL)	7.4 (6.3 – 8.7)	7.3 (6.6 – 10.2)	0.331
Potassium (mEq/L)	4.7±0.5	4.8±0.6	0.255
Phosphorus (mg/dL)	3.7 (3.0 – 3.9)	3.7 (3.2 – 3.9)	0.907
Calcium (mg/dL)	9.6 (9.2 – 9.8)	9.8 (9.4 – 9.9)	0.269
Glucose (mg/dL)	97.0 (91.3 – 104.8)	99.3 (87.3 – 111.0)	0.896
Insulin (uUI/mL)	10.4 (5.3 – 20.3)	10.3 (6.9 – 19.)	0.309
HOMA-IR	3.2 (1.4 – 5.9)	2.6 (1.6 – 5.5)	0.501
Triglycerides (mg/dL)	131.0 (85.3 – 175.8)	122.5 (87.8 – 171.3)	0.886
Total cholesterol (mg/dL)	157.5 (76.5 – 196.5)	179.5 (161.5 – 209.2)	0.059
LDL-cholesterol (mg/dL)	115.6±38.0	104.1±26.2	0.230
HDL-cholesterol (mg/dL)	42.0 (37.0 – 61.0)	45.0 (33.7 – 63.4)	0.973

Expressed as Mean ± SD for normally distributed variables, and as Median (interquartile interval) for non-normal variables, analysed by paired t-test and Wilcoxon signed rank test, respectively.

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance; LDL low-density lipoprotein; HDL, high-density lipoprotein.

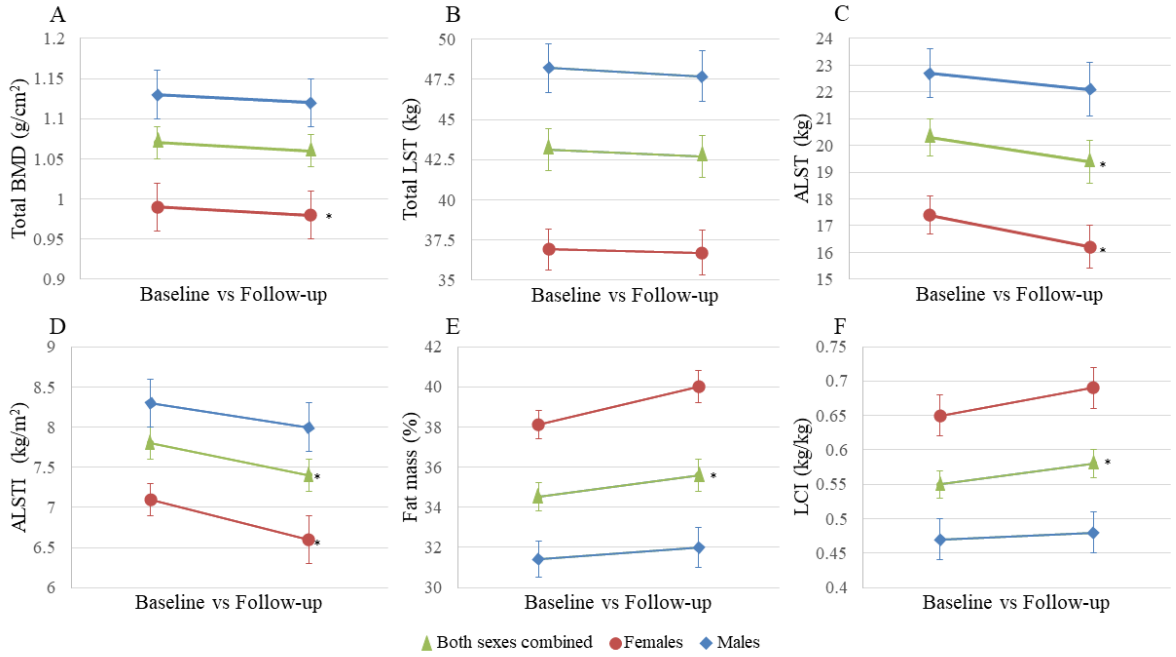

Figure 1. Mean difference in body composition parameters between baseline and follow-up. A) Total bone mineral density (BMD); B) Total lean soft tissue (LST) C) Appendicular LST (ALST); D) ALST index (ALSTI); E) Fat mass; and F) Load-capacity index (LCI) in patients with non-dialysis dependent chronic kidney disease.

Caption: *p<0.05 between baseline and follow-up.
Mean ± SEM for both sexes grouped – BMD: baseline = 1.07±0.02 g/cm^2, follow-up = 1.06±0.02 g/cm^2, p=0.053; Total LST: baseline = 43.1±1.3 kg, follow-up = 42.7±1.3kg, p=0.195; ALST: baseline = 20.3±0.7 kg, follow-up = 19.4±0.8 kg, p=0.031; ALSTI: baseline = 7.8±0.2 kg/h^2, follow-up = 7.4±0.2 kg/h^2, p=0.021; fat mass: baseline = 34.5±1.1 %, follow-up = 35.6±1.1 %, p=0.044; LCI: baseline =0.55±0.03 kg/kg, follow-up = 0.58±0.03, p=0.032.
Mean ± SEM for females – BMD: baseline = 0.99±0.03 g/cm^2, follow-up = 0.98±0.03 g/cm^2, p=0.034; Total LST: baseline = 36.9±1.3 kg, follow-up = 36.7±1.4 kg, p=0.656; ALST: baseline = 17.4±0.7 kg, follow-up = 16.2±0.8 kg, p=0.026; ALSTI: baseline = 7.1±0.2 kg/h^2, follow-up = 6.6±0.3 kg/h^2, p=0.037; fat mass: baseline = 38.1±1.9 %, follow-up = 40.0±1.6 %, p=0.078; LCI: baseline = 0.64±0.05 kg/kg, follow-up = 0.69±0.04 kg/kg, p=0.066.
Mean ± SEM for males – BMD: baseline = 1.13±0.03 g/cm^2, follow-up = 1.12±0.03 g/cm^2, p=0.493; Total LST: baseline = 48.2±1.5 kg, follow-up = 47.7±1.6, p=0.214; ALST: baseline = 22.7±0.9 kg, follow-up = 22.1±1.0 kg, p=0.328; ALSTI: baseline = 8.3±0.3 kg/h^2, follow-up = 8.0±0.3 kg/h^2, p=0.219; fat mass: baseline = 31.4±1.1 %, follow-up = 32.0±1.1 %, p=0.348; LCI: baseline = 0.47±0.02 kg/kg, follow-up = 0.48±0.02 kg/kg, p=0.289.

Table 2. Changes in body composition parameters between baseline and follow-up, and comparison between normal and low BMD groups in patients with non-dialysis dependent chronic kidney disease.

Parameter	Group	Baseline [a]	Follow-up [a]	% Change	P [b]
BMD (g/cm^2)	Normal BMD	1.16±0.10	1.15±0.11	-0.83	0.080
	Low BMD	0.93±0.10†	0.93±0.11†	-0.55	0.398
Total LST (kg)	Normal BMD	45.6±9.5	45.6±9.8	-0.05	0.987
	Low BMD	39.4±6.5$^{\#}$	38.6±6.0$^{\#}$	-2.02	**0.041**
ALST (kg)	Normal BMD	21.6±4.7	21.0±5.6	-2.55	0.339
	Low BMD	18.5±3.7*	17.0±3.4$^{\#}$	-7.35	**0.006**
ALSTI (kg/m^2)	Normal BMD	8.1±1.4	7.8±1.6	-2.95	0.276
	Low BMD	7.3±1.3	6.7±1.1$^{\#}$	-7.05	**0.008**
Total fat mass (%)	Normal BMD	36.5±6.7	37.0±7.3	1.34	0.364
	Low BMD	31.7±8.4*	33.8±7.7	9.08	0.074
LCI (kg/kg)	Normal BMD	0.59±0.18	0.61±0.19	2.79	0.229
	Low BMD	0.49±0.18	0.53±0.18	14.05	0.082

Expressed as mean ± SD, all variable normally distributed.

[a] Independent t-test at each time point; *$p<0.05$, $^{\#}p<0.01$, and $^{\dagger}p<0.001$ in Low BMD compared to normal BMD.

[b] Paired t-test baseline vs follow-up; bolded values indicate significant difference ($p<0.05$), only observed in Low BMD.

Abbreviations: BMD, bone mineral density; LST, lean soft tissue; ALST, appendicular LST; ALSTI, ALST index; LCI, load-capacity index.

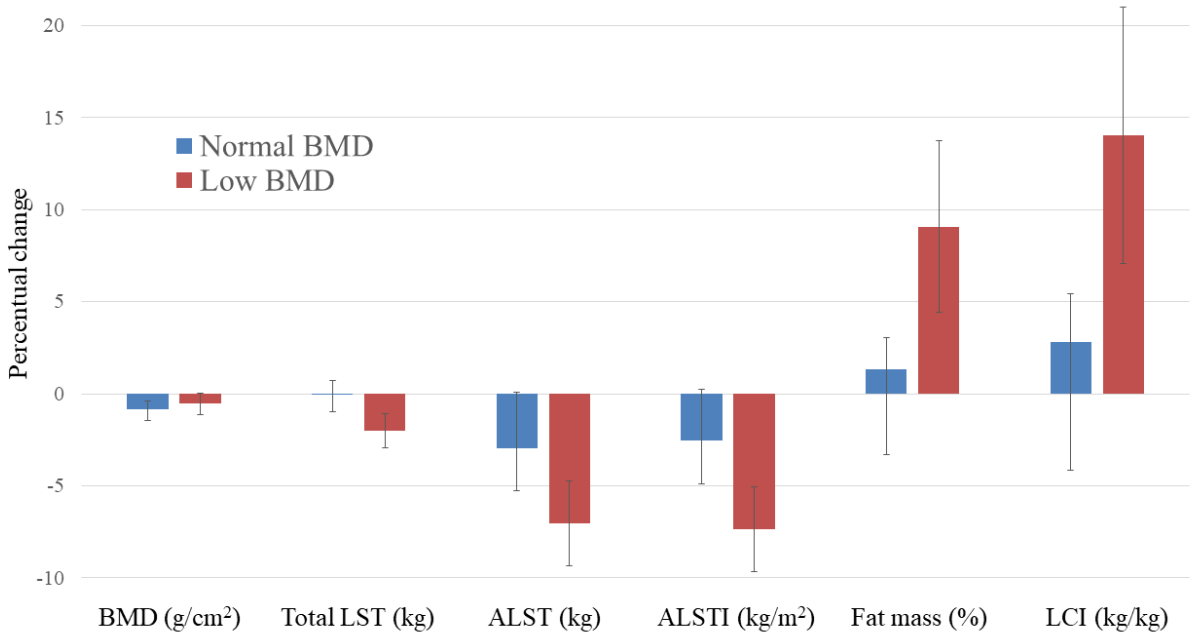

Figure 2. Percentual changes in body composition parameters according to bone mineral density (BMD) classification at baseline (normal: t-score > -0.1; low: t-score ≤ -1.0) in patients with non-dialysis dependent chronic kidney disease.

Caption: Variables normally distributed expressed as mean ± SEM, variables not normally distributed expressed as median (interquartile interval). BMD (g/cm^2): normal BMD = -0.83±0.45, low BMD = -0.55±0.60, p=0.708; total lean soft tissue (LST, kg): normal BMD = -0.05±0.77, low BMD = -2.02±0.94, p=0.109; appendicular LST (ALST, kg): normal BMD = -2.95±3.03, low BMD = -7.05±2.37, p=0.221; ALST index (ALSTI, kg/m^2): normal BMD = -2.55±2.08, low BMD = -2.55±2.8, p=0.326; fat mass (%): normal BMD = 0.89 (-1.88 – 5.14), low BMD = 3.00 (-4.00 – 19.78), p=0.400; load-capacity index (LCI): normal BMD = 1.76 (-2.83 – 8.68), low BMD = 5.24 (-6.01 – 28.51), p=0.441.

Table 3. Odds ratio (OR) and 95% confidence interval (95% CI) for low bone mineral density (BMD) at follow-up according to the presence of possible risk factors related to body composition at baseline in patients with non-dialysis dependent chronic kidney disease.

Low BMD risk factors	**Model 1**			**Model 2**			**Model 3**			**Model 4**		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Total LST (kg)	0.899	0.829, 0.976	**0.010**	0.770	0.639, 0.929	**0.006**	0.777	0.642, 0.942	**0.010**	0.778	0.643, 0.941	**0.010**
FM (%)							0.881	0.760, 1.021	0.093			
LCI (kg/kg)										0.007	0.000, 2.468	0.097
ALST (kg)	0.825	0.704, 0.967	**0.017**	0.564	0.384, 0.828	**0.004**	0.593	0.405, 0.867	**0.007**	0.593	0.406, 0.866	**0.007**
FM (%)							0.881	0.750, 1.034	0.121			
LCI (kg/kg)										0.008	0.000, 4.268	0.131
ALSTI (kg/m^2)	0.586	0.354, 0.968	**0.037**	0.182	0.048, 0.685	**0.012**	0.204	0.056, 0.752	**0.017**	0.210	0.058, 0.761	**0.017**
FM (%)							0.873	0.739, 1.031	0.110			
LCI (kg/kg)										0.006	0.000, 4.939	0.135

Model 1: unadjusted; Model 2: adjusted for estimated glomerular filtration rate (eGFR), sex, age, and time between baseline and follow-up; Model 3: adjusted for eGFR, sex, age, time, and fat mass (FM); Model 4: adjusted for eGFR, sex, age, time, and load-capacity index (LCI). The goodness-of-fit was assessed by Homer-Lemeshow test, which did not present collinearity between variables.

Abbreviations: BMD, bone mineral density; LST, lean soft tissue; ALST, appendicular LST; ALSTI, ALST index

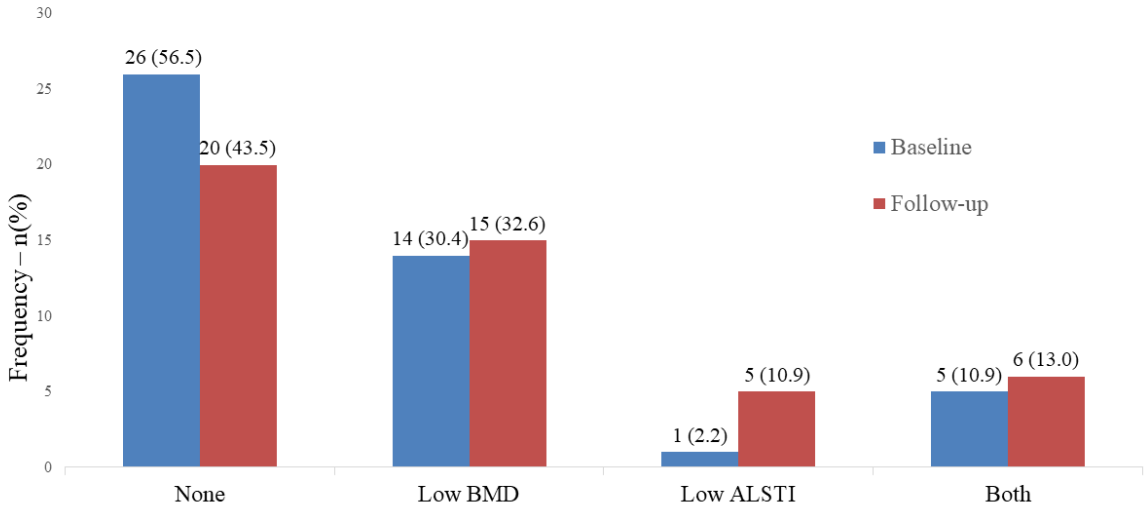

Figure 3. Frequency (n and %) of low bone mineral density (BMD), low muscle mass by appendicular lean mass index (ALSTI), and both conditions (low BMD and low ALSTI) in patients with non-dialysis dependent chronic kidney disease at baseline and follow-up.

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