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Research Article

The role of osteopontin and osteocyte-derived factors in secondary hyperparathyroidism-induced myopathy

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Abstract

Muscle weakness is a common symptom in CKD patients, and the pathway by which secondary hyperparathyroidism (SHPT) affects muscle function is unknown. Osteopontin (OPN), a bone matrix protein stimulated by PTH and phosphate, has been associated with inflammatory muscle diseases. In this observational and prospective cohort study, we evaluated 30 patients with severe SHPT (39 ± 12 yr; 18 women), before and 6 mo after parathyroidectomy (PTx). We examined the relationships among CKD–mineral and bone disorder parameters; myokine and inflammatory cytokine levels; and changes in resting energy expenditure (REE), muscle function, BMD, and muscle-related proteins. At baseline, the patients showed low gene expression of muscle turnover markers and irisin, as well as high protein expression of OPN, transforming growth factor beta (TGF-β), and fibroblast growth factor 21. Six months after PTx, REE and muscle mass had not changed, but physical performance, muscle strength, and bone mass improved, more so in patients undergoing total PTx. Also, there were reductions in the protein expression of OPN (11 vs 3%, p=.01) and TGF-β (21 vs 7%, p=.002) in muscle, together with a significant increase in irisin muscular levels (30 vs 35 pg/mg, p=.02). The gain in bone mass and the increase in irisin levels correlated with a reduction in PTH. The levels of interleukin (IL)-1β, tumor necrosis factor alpha, and IL-17 (markers of myositis) were also lower after PTx. Our data suggest that SHPT plays a role in CKD-induced muscle dysfunction, indirectly, via release of bone-specific proteins, which is partially reverted with PTx.

Keywords: inflammation, muscle, osteopontin, parathyroidectomy, TGF-β

Lay Summary

How secondary hyperparathyroidism (SHPT) affects muscle quality in CKD patients is unclear. This observational, prospective study performed muscle phenotyping by performance evaluation, body composition analysis, and muscle biopsies of 30 patients on dialysis with SHPT, before and after parathyroidectomy (PTx). The main findings include evidence of local tissue inflammation and blunting of muscle turnover in patients compared to healthy individuals. Despite no changes to muscle mass or energy expenditure, PTx improved functionality, bone mass, and influenced the molecular phenotyping of muscle tissue by altering the expression of bone-specific proteins and reducing inflammation. These findings suggest that SHPT plays a role in CKD-induced muscle dysfunction and that PTx has a beneficial effect on uremic myopathy.

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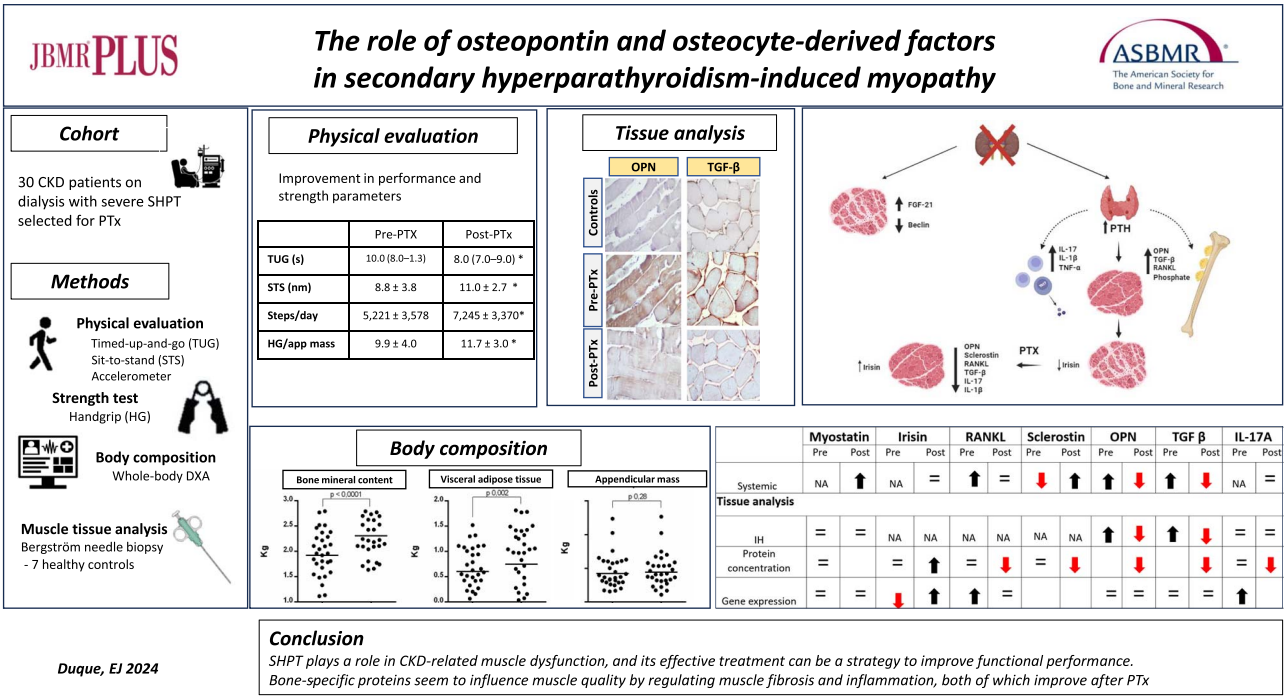
Graphical Abstract



Introduction

Advanced secondary hyperparathyroidism (SHPT) is associated with a high risk of extraskeletal calcification, decreased cortical bone density, and muscle dysfunction.[1] In CKD, the cause of muscle disability is multifactorial, resulting from an imbalance between muscle synthesis and proteolysis, as well as from the effects of uremic toxins, mitochondrial dysfunction, inflammation, insulin resistance, and vitamin D deficiency.[2]

Sarcopenia is characterized by a loss of muscle mass and impaired functionality. In experimental models of CKD, PTH has been shown to be a potential mediator of muscle loss through adipose tissue browning.[3] Moreover, parathyroidectomy (PTx) has been shown to increase muscle strength in patients with primary hyperparathyroidism.[4]

There is evidence that bone and muscle communicate, mechanically and biochemically.[5] Irisin is a peptide released by muscle after cleavage of FNDC5 (fibronectin type III domain-containing protein 5) during exercise, and it has been shown to have effects on bone tissue[6] and to correlate negatively with PTH.[7] Experimental studies have suggested that other myokine, the fibroblast growth factor 21 (FGF21), mediates not only muscle atrophy and weakness,[8] but negatively affects bone tissue.[9] Osteopontin (OPN), a bone matrix protein encoded by the secreted phosphoprotein 1 (*SPP1*) gene, is stimulated by PTH and phosphate; in fact, OPN modulates inflammatory responses in several diseases,[10] including muscular dystrophy, through regulation of intramuscular transforming growth factor beta (TGF-β).[11] Some osteocyte-derived factors, such as RANKL and sclerostin, are also involved in the pathogenesis of muscle diseases.[12] The role of SHPT in muscle dysfunction is not completely understood. In addition to its direct effect, excessive PTH can affect muscle indirectly through altered bone remodeling. That said, we here hypothesized that PTx can ameliorate muscle and bone disorders in patients under hemodialysis. Therefore, the aim of this study was to examine how SHPT and PTx affect muscle phenotype in CKD patients, and their influence on the expression of bone-specific proteins in muscle tissue.

Materials and methods

Study sample and biochemical measurements

This is a longitudinal, observational, prospective cohort study of CKD patients ≥18 yr of age who were on hemodialysis and being followed at the CKD–mineral and bone disorder (CKD-MBD) outpatient clinic of the Nephrology Division of the Hospital das Clínicas, in São Paulo, Brazil. Patients with serum levels of intact PTH greater than 800 pg/mL on calcitriol or in the presence of hyperphosphatemia and/or hypercalcemia, which prevented the use of calcitriol, were considered as having refractory hyperparathyroidism, referred to PTx and invited to participate. Total with autotransplantation or subtotal PTx was done at the discretion of surgery team. Non-eligibility criteria included being on peritoneal dialysis; physical limitations (walking aid required); prior exposure to medications affecting bone metabolism, such as bisphosphonates, steroids, and other immunosuppressants; diabetes; high cardiovascular risk; and infectious diseases (hepatitis or HIV).

Fasting blood samples were collected from all participants before and 6 mo after PTx, by which time the postoperative hungry bone syndrome has typically stabilized. PTH and 25OHD were measured by chemiluminescent immunoassay (Roche Diagnostics), as were bone turnover markers, such as C-terminal telopeptide of type 1 collagen (CTx) and P1NP. Diasorin assay was used to measure 1,25-dihydroxyvitamin D (1,25[OH]$_2$D). Sclerostin, RANKL, intact FGF23, osteocalcin, osteoprotegerin (OPG), OPN, leptin, interleukin (IL)-6, IL-1β, IL-17A, TNF-α, TGF-β, and myokines (myostatin, irisin, and FGF21) were measured with a commercial kit (MILLIPLEX Human

Bone Metabolism Magnetic Bead Kit; Merck Millipore). The study protocol was approved by Research Ethics Committee of the Hospital das Clínicas (# 87886218.7.0000.0068), and all participating patients and healthy subjects gave written informed consent. In addition, muscle strength [by handgrip analysis], physical performance tests [GT3X accelerometer, time-up-and-go (TUG) and sit-to-stand (STS)], resting energy expenditure (REE) [by indirect calorimetry], and body composition (by DXA) assessments were performed in all participants before and 6 mo after PTx. A detailed description is provided in the Supplementary Methods.

Muscle biopsies

Immediately before and 6 mo after PTx, muscle biopsy samples were extracted from the vastus lateralis with a modified Bergström needle. The biopsy fragments were sent for gene and protein expression analysis. Control biopsies were taken in 7 (4F:3M) healthy age-matched subjects with unremarkable screening biochemical tests.

Histological preparation and immunohistochemical staining

For immunohistochemical evaluation, muscle tissue sections were fixed in 10% neutral-buffered formalin and processed in an automatic tissue processor (Jung Histokinette 2000; Leica Instruments GmbH, Nussloch, Germany). Paraffin-embedded tissues were cut into 5-μm sections and mounted on slides. Sections were submitted to heat-mediated antigen retrieval, after which we performed immunoperoxidase staining, using an EnVision+ System, HRP kit (Dako/Agilent Technologies) to identify atrogin (ab157596; Abcam), myostatin (PA5-11936; Invitrogen), beclin 1 (PB9076; Boster Bio), and TGF-β (GTX21279; GeneTex), as well as a Vectastain ABC-HRP kit (PK4000; Vector Laboratories) to identify vitamin D receptor (VDR) (MA1-710; Invitrogen) and OPN (SC10593; Santa Cruz Biotechnology). In all experiments, negative controls were obtained by omitting the primary antibody. Atrogin, myostatin, OPN, and TGF-β were quantified by a point-counting method, with a 144-point grid, and the results are expressed as percentages of the total area of the muscle section. We quantified VDR by counting labeled cells in muscle tissue. Muscle protein concentration and gene expression analysis are described in Supplementary Methods.

Statistical analysis

Continuous variables were expressed as means and standard deviations or as medians and interquartile ranges, as appropriate. Continuous variables were compared with a paired *t*-test or the Wilcoxon test for those with Gaussian and non-Gaussian distribution, respectively. Categorical variables are expressed as absolute values and percentages; the pre- and post-PTx values were compared by McNemar's test. Correlations between independent variables were quantified by calculating Pearson's or Spearman's coefficient, as appropriate. Values of $p<.05$ were considered statistically significant. Analyses were performed with the IBM SPSS Statistics software package, version 21.0 (IBM Corporation) and GraphPad Prism software, version 9.0 (GraphPad Software, Inc.).

Results

Between July 2018 and December 2021, 63 adult patients with CKD underwent PTx in our center. As shown in Figure S1, 32 patients met the eligibility criteria and were included to undergo baseline muscle biopsy, DXA, muscle function tests, indirect calorimetry for the assessment of REE, and blood tests. Two patients were excluded thereafter. Of the 30 patients in the final sample, 18 (60%) were women. The mean age was 39 ± 12 yr, and the median dialysis vintage was 66 mo (Table 1). Twenty patients underwent subtotal PTx, and 10 underwent total PTx with forearm autotransplantation.

Six months after PTx, PTH, calcium, phosphate, alkaline phosphatase, CTX, and P1NP reduced significantly (Table 2). Moreover, the PTH level was significantly lower in the patients who underwent total PTx than in those who underwent subtotal PTx (54 vs 231 pg/mL, p=.02; respectively). Concomitantly, after 6 mo of PTx, the 25OHD and 1,25$(OH)_2$D increased, while serum FGF23, DKK1, OPG, and OPN reduced, together with an increase in sclerostin. No significant changes were observed in RANKL and osteocalcin.

There were significant post-PTx increases in the BMI, in the Homeostatic Model Assessment for Insulin Resistance index, IGF-1, and glycated hemoglobin, together with a significant decrease in leptin (Table 1). We found no significant changes in fasting glucose, total cholesterol, or triglycerides. Although circulating levels of irisin and FGF21 did not change after PTx, there was an increase in serum myostatin and a reduction in serum TGF-β. There were also post-PTx decreases in C-reactive protein and IL-6, although there were no changes in IL-1β, IL-17A, or TNF-α (Table 2).

REE, muscle function, and physical performance tests

Six months after PTx, the patients showed improvement in handgrip strength, number of steps/day, as well as better performance on the TUG and STS tests (Table S1). There was no difference between the pre- and post-PTx REE.

Changes in BMD and body composition

Table 3 shows the comparisons between pre- and 6 mo post-PTx regarding BMD parameters and body composition. At baseline, 8 patients (26.7%) had low muscle appendicular mass index or low total muscle appendicular mass. In 6 patients, the low muscle mass persisted at 6 mo after PTx (p=.5). Seven patients (23.3%) had a *T*-score ≤ -2.5, and 14 patients (46.7%) had a *T*-score between -1.1 and -2.4 at one or more bone sites. There were significant gains in bone mass, overall, in the LS, and in the femur. In addition to the bone mass gain, there were significant increases in fat mass and fat percentage, at the expense of a gain in visceral adipose tissue, with no impact on appendicular mass.

The improvement in BMD was significantly greater in patients with final PTH < 80 pg/mL than in those with PTH ≥ 80 pg/mL (Figure 1). The drop in PTH levels correlated with improvement in bone mass and BMD. There was also a negative correlation between the PTH level and fat mass gain.

Gene expression in muscle tissue

At baseline, expression of *RANKL* in muscle was 16 times higher in the patients than in the controls (Table S2). Expression of *FGF21* was also higher (p=.06), whereas that of *AKT* was slightly lower (p=.058). However, there were no post-PTx differences. Although the baseline expression of *FNDC5* was lower in the patients (p=.03), that expression doubled after PTx (Figure 2). Baseline expression of *FOXO3* was also lower in the patients than in the controls. No differences were noted

Table 1. Baseline characteristics and metabolic parameters before and 6 mo after parathyroidectomy ($N = 30$).

Variable	Pre-PTx	Post-PTx	*p*
Age (years)	39 ± 12		
Female, *n* (%)	18 (60.0)		
Dialysis vintage (months)	66 (56–95)		
Etiology of CKD, *n* (%)			
Glomerulonephritis	16 (53.3)		
Hypertension	6 (20.0)		
Idiopathic	4 (13.3)		
ADPKD	2 (6.6)		
Urologic	2 (6.6)		
Calcium carbonate	6 (20)	23 (82)	
Vitamin D analogs	12 (40)	21 (75)	
Calcimimetic agent	24 (80)	0	
Sevelamer	27 (90)	7 (25)	
BMI (kg/m^2)	25.8 ± 3.9	26.6 ± 4.0	**.001**
CRP (mg/dL)	6.4 (4.0–12.0)	3.6 (2.0–8.0)	**.01**
Albumin (g/dL)	4.5 ± 0.4	4.6 ± 0.3	.08
Hemoglobin (g/dL)	11.3 ± 1.6	12.6 ± 1.7	**.001**
Glycemia (mg/dL)	85 (73–90)	84 (72–90)	.345
HbA1C (%)	5.10 ± 0.48	5.30 ± 0.53	**.008**
HOMA-IR index	1.74 (0.8–3.1)	2.29 (1.4–5.8)	**.006**
Cholesterol (mg/dL)	150 (137–172)	167 (147–187)	.2
Triglycerides (mg/dL)	128 (98–162)	133 (99–186)	.3
Bicarbonate (mmol/L)	22.3 (21.6–24.2)	23.0 (21.2–24.9)	.91
IGF-1 (ng/mL)	173 (139–218)	197 (151–260)	**.04**
GH (ng/mL)	0.36 (0.10–1.40)	0.56 (0.20–1.40)	.3
Leptin (ng/mL)	18.6 (7.8–81.1)	0.78 (0.6–1.1)	**.0007**

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; CRP, C-reactive protein; GH: growth hormone; HbA1C, glycated hemoglobin; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, IGF-1, insulin-like growth factor 1; PTx, parathyroidectomy. Data are presented as mean ± SD, *n* (%), or median (IQR). Bold data indicate significance.

Table 2. Serum biochemical parameters, bone-related proteins, myokines, and cytokines, at baseline and 6 mo after parathyroidectomy ($N = 30$).

Parameter	Pre-PTx	Post-PTx	*Changes, absolute/%*	*p*
Calcium (mg/dL)	9.6 (8.7–10.5)	8.6 (7.5–9.0)	−1/−10.4	**.002**
Phosphate (mg/dL)	6.0 ± 1.6	4.8 ± 1.1	−1.2/−20	**.0001**
ALP (U/L)	373 (212–736)	83 (65–131)	−290/77.7	**.0001**
25OHD (ng/mL)	27 (18–36)	39 (28–48)	12/44.4	**.002**
1,25(OH)$_2$D (pg/mL)	8.75 (5.98–12.50)	14.60 (8.76–23.50)	5.8/66.8	**.007**
PTH (pg/mL)	1526 (1380–1959)	119 (36–297)	−1407/92.2	**.0001**
FGF23 (pg/mL)	1451 (1030–2035)	799 (184–1667)	−652/−44.9	**.001**
DKK1 (pg/mL)	1396 ± 541	1003 ± 337	−393/−28.1	**.001**
Sclerostin (ng/mL)	2.16 (1.18–2.64)	4.15 (2.25–5.58)	2/92.1	**.01**
CTX (ng/mL)	6.0 (5.0–6.0)	1.4 (0.6–2.4)	−4.6/−76.6	**.01**
P1NP (ng/mL)	2400 (1262–2400)	392 (189–707)	−2008/−83.6	**.01**
Osteocalcin (ng/mL)	89 (63–133)	106 (55–150)	17/19.1	.56
OPN (ng/mL)	37.8 (17–68)	12.5 (3.7–21)	−25.3/−66.9	**.0001**
RANKL (pg/mL)	14.8 (6.1–35.0)	18.3 (6.7–32.0)	3.5/23.6	.17
OPG (pg/mL)	789 (642–1092)	739 (469–928)	−50/−6.3	**.02**
Myostatin (pg/mL)	519 (271–823)	653 (480–1173)	134/25.8	**.02**
Irisin (pg/mL)	109 (92–156)	128 (92–183)	19/17.4	.1
FGF21 (pg/mL)	447 (67–745)	227 (118–764)	−220/−49.2	.7
TGF-β (ng/mL)	21.8 ± 12.0	13.0 ± 9.0	−8.8/−40.3	**.003**
IFN-γ (pg/mL)	11.1 ± 6.2	11.6 ± 5.5	0.5/4.5	.47
IL-1β (pg/mL)	1.16 (0.8–1.6)	1.11 (0.7–1.9)	−0.05/−4.3	.62
IL-6 (pg/mL)	11.4 ± 25.0	8.7 ± 21.0	−2.7/23.6	**.02**
IL-17A (pg/mL)	7 (3.7–10.4)	7 (3.9–10.5)	0	.76
TNF-α (pg/mL)	18 (13–23)	17 (12–24)	−1/−5.5	.96

Abbreviations: 1,25 (OH)$_2$D, 1,25-dihydroxyvitamin D; ALP, alkaline phosphatase; CTX, C-terminal telopeptide of collagen type 1; DKK1, dickkopf 1; FGF, fibroblast growth factor; IFN, interferon; IL, interleukin; OPG, osteoprotegerin; OPN, osteopontin; PTx, parathyroidectomy; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha. Data are presented as mean ± SD or median (IQR). Bold data indicate significance. Reference values: Calcium, 8.4–10.2 mg/dL; Phosphate, 2.3–4.7 mg/l; ALP, 46–122 U/L; 25OHD, 30–60 ng/mL for risk groups, including CKD patients; 1,25 (OH)$_2$D, 19.9–79.3 pg/mL; CTX, ≤ 0.7 and ≤ 0.57 ng/mL for men and women 50–70 yr of age, respectively; P1NP, 13.9–85.5 ng/mL for men and 15.1–58.6 ng/mL for women; PTH, 15–65 pg/mL.

Table 3. Body composition at baseline and 6 mo after parathyroidectomy ($N = 30$).

Variable	Pre-PTx	Post-PTx	*Changes, absolute/%*	*p*
BMD				
LS (g/cm^2)	1.05 ± 0.19	1.22 ± 0.21	0.17/16.2	**<.001**
TH (g/cm^2)	0.86 ± 0.17	1.02 ± 0.17	0.16/18.6	**<.001**
FN (g/cm^2)	0.79 ± 0.18	0.97 ± 0.18	0.18/22.8	**<.001**
***T*-score, points (women;men)**				
>−1 SD	9 (5;4)	16 (8;8)		**.002**
−1.1 to −2.4	14 (7;7)	12 (9;3)		
≤−2.5 SD	7 (6;1)	2 (1;1)		
BMC (g)	1958 (1654–2309)	2236 (2080–2696)	278/14.1	**<.001**
Lean mass (g)	40 375 (37 717–48 213)	40 091 (37 552–46 688)	−284/−0.7	.17
Fat mass (g)	23 521 ± 8.88	26 196 ± 9.34	2675/11.3	**.001**
Fat (%)	34.1 ± 10.8	36.8 ± 9.5	2.7/7.9	**.001**
SMI (kg/m^2)	7.08 ± 1.20	7.22 ± 1.10	0.14/1.9	.16
Appendicular mass (g)	17 397 (15 828–22 101)	18 312 (16 346–21 617)	915/5.2	.28
VAT (g)	753 ± 457	1003 ± 556	250/33.2	**.002**

Abbreviations: PTx, parathyroidectomy; SMI, skeletal mass index; VAT, visceral adipose tissue. Data are presented as mean ± SD or median (IQR), except where otherwise indicated. Bold data indicate significance. Reference values: SMI, ≥ 5.5 kg/m^2 for women and ≥ 7 kg/m^2 for men.

regarding expression of *IGF-1*, *VDR*, *MSTN*, *FOXO1*, *TNF-α*, *IL-6*, *RANK*, *KL*, or *FGF23*.

Protein concentration and expression in muscle tissue

We observed significant post-PTx decreases in muscular protein concentration for cytokines (IL-1β, IL-17A, and TNF-α), OPN, RANKL, TGF-β, and sclerostin (Table 4). On immunohistochemistry (Figure 3), we observed an increase in VDR expression, together with reductions in the expression of TGF-β (p=.002) and OPN (p=.01). There were no post-PTx changes in the expression of beclin, atrogin, or myostatin. There was a significant association between the increase in number of steps per day and the percentual change in the muscular concentration of OPN ($r = -0.697$, p=.01) and FGF23 ($r = -0.721$, p=.003).

As illustrated in Figure 4, the post-PTx increase in muscle irisin concentration was significantly (12-fold) greater in the patients with final PTH < 80 pg/mL than in those with PTH ≥ 80 pg/mL (p=.006). The muscle irisin concentration correlated positively with bone mass gain ($r = 0.547$, p=.01) and spinal BMD ($r = 0.507$, p=.02), whereas it correlated negatively with tissue expression of TGF-β ($r = -0.446$, p=.04).

Discussion

We investigated the effect of SHPT and PTx on muscle phenotypes in CKD patients on dialysis, assessing muscle function and body composition, as well as muscle-related gene and protein expression. We found that SHPT induced histological changes related to mitochondrial dysfunction, promoted fibrosis, and blunted the expression of markers of muscle turnover. In addition to bone mass gain and improved functionality, PTx also reduced tissue expression of OPN and TGF-β, as well as reducing inflammation, despite having no apparent effect on muscle mass.

In CKD patients, PTH acts as a uremic toxin due to its deleterious effects on various systems.[1] Severe bone disease, often accompanied by fractures and vascular calcifications, occurs when clinical treatment is not effective, sometimes due to delayed diagnosis of SHPT. Studies have shown that survival improves when patients with moderate or severe SHPT undergo PTx.[13] Beyond its beneficial effects on bone tissue, PTx improves nutritional status. Although there were no changes in REE after PTx, we noticed a significant increase in fat mass, at the expense of visceral fat, together with higher insulin resistance, suggesting a shift toward a metabolic syndrome profile in these patients.

A significant post-PTx gain in BMD has been widely reported, particularly in patients with primary hyperparathyroidism but also in patients on hemodialysis.[14-16] In the present study, we noticed that the post-PTx gain in BMD was approximately 90% greater in the patients with final PTH < 80 pg/mL. Our finding underscores the impact that effective control of PTH has on bone mass.

The effects of PTx on bone histomorphometry have been analyzed previously.[17] Studies have shown that bone turnover and structural parameters improve after PTx, whereas mineralization worsens and expression of sclerostin increases, as does that of OPG. The post-PTx reduction in PTH has been shown to suppress bone resorption and accelerate bone mineralization.[18] In our cohort, the reduction in PTH blunted the release of the bone remodeling markers CTX and P1NP. As expected, the suppression of PTH ramped up serum sclerostin. However, there was a significant reduction in its muscle concentration, showing a disagreement between systemic and local concentrations of sclerostin.

Other osteocyte-derived proteins, such as RANKL, are involved in muscle function. Inhibition of RANKL/RANK has been shown to improve muscle strength in murine models of muscular dystrophy.[19] That effect has been also investigated in different models of chronic diseases. In animal models of chronic obstructive pulmonary disease, RANKL concentrations in skeletal muscle fibers have been found to be elevated, and RANKL neutralization has been shown to restore muscle strength and function.[20] In a 3-yr clinical study, anti-RANKL therapy was shown to be superior to treatment with bisphosphonates or no treatment, for improving appendicular lean mass and handgrip strength in postmenopausal women.[21] In our cohort, there was a post-PTx reduction in serum OPG levels and no change in circulating RANKL. In the histological analysis, we noticed that baseline *RANKL* expression was higher in the patients

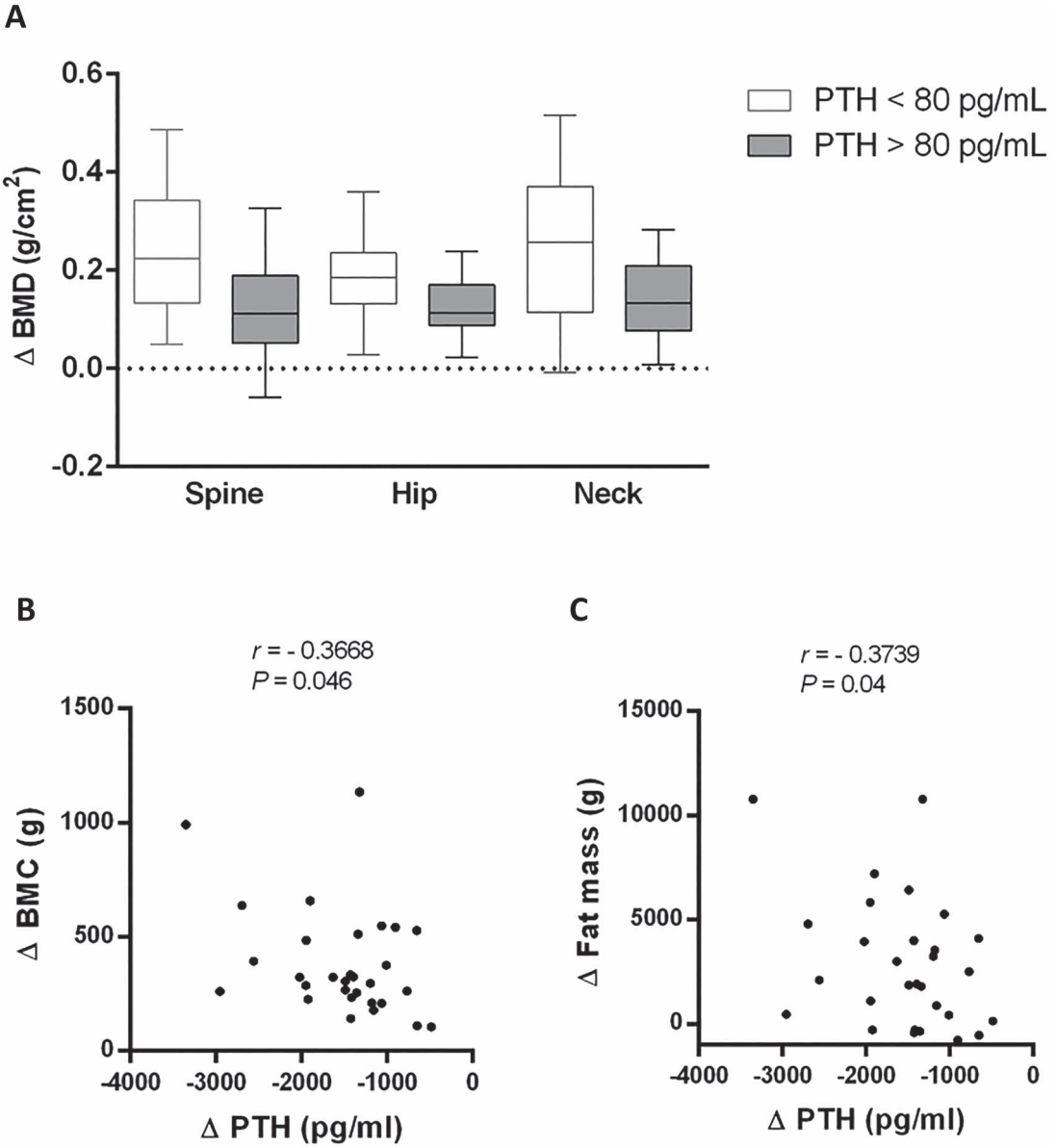


Figure 1. Changes in DXA absorptiometry parameters after PTx and their association with the post-PTx change in PTH. (a) Variation in BMD according to the final PTH. (b) Correlation between the variation in PTH levels and the variation in BMC. (c) Correlation between the variation in PTH levels and the variation in fat mass.

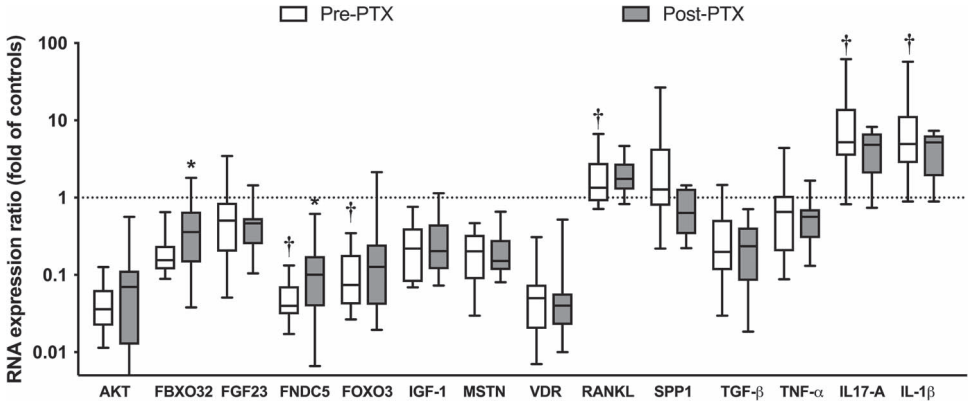


Figure 2. Relative muscle tissue gene expression at baseline and 6 mo after PTx. The upper and lower whiskers represent the 10th and 90th percentiles. *$p < .05$ vs pre-PTx; †$p < .05$ vs controls.

Table 4. Results of multiplex protein quantification and immunohistochemistry to quantify protein expression in muscle tissue collected from patients ($N = 30$), at baseline and 6 mo after parathyroidectomy, and from healthy age-matched controls ($N = 7$).

Protein	Controls	Pre-PTx	Post-PTx	p[a]
Quantification				
TGF-β (pg/mg)	2.50 (1.60–3.20)	2.84 (1.90–5.60)	2.72 (2.10–3.00)	**.04**
Irisin (pg/mg)	33 (26–35)	30 (22–48)	35 (29–152)	**.02**
Myostatin (pg/mg)	15 (12–20)	15 (12–24)	13.4 (12–27)	.91
FGF21 (pg/mg)	0.01 (0.01–0.02)	0.03 (0.02–0.06)[b]	0.02 (0.02–0.05)[b]	.71
FGF23 (pg/mg)	1.78 (1.23–2.45)	2.3 (1.3–4.1)	1.8 (1.5–2.4)	.10
Sclerostin (pg/mg)	2.06 (1.54–2.72)	2.01 (1.58–5.28)	1.82 (1.21–2.26)	**.008**
DKK1 (pg/mg)	0.34 (0.11–0.43)	0.39 (0.27–1.07)	0.37 (0.18–0.72)	.82
OPN (pg/mg)	47.4 (35.0–119.0)	179 (103.0–505.0)[b]	54 (37.0–142.0)	**.01**
OPG (pg/mg)	0.34 (0.25–0.42)	0.43 (0.3–0.74)	0.35 (0.28–0.84)	.83
Osteocalcin (pg/mg)	9.99 (8.40–18.10)	262.0 (44.0–602.0)[b]	37.0 (22.0–72.0)	**.01**
GM-CSF (pg/mg)	1.07 (0.76– 2.51)	2.14 (0.92–4.81)	1.04 (0.72–2.48)	.05
RANKL (pg/mg)	42 ± 25	52 ± 36	30 ± 13	**.03**
IFN-γ (pg/mg)	0.22 (0.14–0.34)	0.3 (0.16–0.55)	0.24 (0.13–0.34)	.12
IL1-β (pg/mg)	0.32 (0.2–0.47)	0.2 (0.15–1.2)	0.18 (0.13–0.24)	**.001**
IL-6 (pg/mg)	0.01 (0.004–0.01)	0.01(0.007–0.02)	0.01 (0.006–0.01)	.33
IL-17A (pg/mg)	0.16 (0.14–0.27)	0.27 (0.16–1.66)	0.20 (0.11–0.52)	**.03**
TNF-α (pg/mg)	0.05 (0.03–0.06)	0.04 (0.03–0.06)	0.03 (0.02–0.05)	**.04**
pAKT/tAKT (MFI)	18.6 (9.0–764.0)	1.9 (1.1–5.7)[b]	4.1 (2.4–14.2)[b]	**.01**
Immunostaining				
VDR (cells/mm^2)	39 ± 28	34 ± 14	78 ± 28[b]	**.002**
Myostatin (%)	18 ± 2	16 ± 4	20 ± 7	.12
TGF-β (%)	2.3 (1.4–2.6)	21 (16–28)[b]	7 (6–11)	**.002**
OPN (%)	2.9 (1.0–6.8)	11.0 (9.0–16.0)[b]	3.0 (1.0–5.0)	**.01**
Beclin-1 (%)	50.0 ± 3.0	40.7 ± 7.0[b]	43.5 ± 10.0	.40
Atrogin (%)	6.4 (1.5–15.0)	3.1 (1.9–7.4)	2.9 (1.5–5.4)	.39

Data are presented as mean ± SD or median (IQR). Bold data indicate significance. [a]Post-PTx vs baseline; [b]$p < .05$ vs controls; Abbreviations: %, stained proportion of the total area of tissue evaluated; DKK1, dickkopf 1; FGF, fibroblast growth factor, GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MFI, mean fluorescence intensity; OPN, osteopontin; OPG, osteoprotegerin; pAKT, phosphorylated serine/threonine kinase (Ser473); PTx, parathyroidectomy; tAKT, total serine/threonine kinase; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; VDR, vitamin D receptor.

than in the controls. Protein expression of RANKL in muscle dropped after PTx, whereas that of OPG was unchanged. That suggests that local RANKL plays a role in the development of SHPT-induced myopathy.

The post-PTx reduction in the muscular concentration of OPN accompanied the reduction in bone turnover after PTx. In addition to its effect on bone remodeling and mineralization, OPN is a known regulator of inflammatory response by attracting macrophages[22] and inducing synthesis of pro-inflammatory cytokines.[23] In an animal model of muscular dystrophy, knockout of muscle OPN was shown to promote a pro-regenerative tissue phenotype and to delay disease progression.[24] Levels of OPN have also been found to correlate significantly with T-helper 17 differentiation and IL-17 levels in the synovial fluid of patients with rheumatoid arthritis.[25] In addition to inflammatory modulation, OPN has been shown to correlate with intramuscular TGF-β expression.[11] In the present study, there were significant post-PTx reductions in the muscle expression of OPN and TGF-β. Produced mainly by osteocytes, TGF-β signaling is required for the mechanosensitive regulation of sclerostin.[26] Therefore, the decrease in muscle TGF-β after PTx could also explain the local decrease in sclerostin in our patients, despite lower levels of systemic PTH. TGF-β is also stored in the bone matrix and plays a role in bone remodeling by regulating osteoclast and osteoblast recruitment. In patients with SHPT, bone marrow expression of TGF-β has been shown to be elevated before PTx and to normalize thereafter.[27] In pathological conditions, such as metastatic cancer, TGF-β plays a direct role in skeletal muscle weakness. In metastatic cancer, the increased circulating levels of TGF-β after bone resorption induced by metastases have been shown to affect muscle proteins and calcium receptors, contributing to muscle weakness.[28] In our cohort, serum and muscle TGF-β decreased after PTx, which could explain the improvement in muscle responsiveness, given that TGF-β promotes tissue fibrosis.

There is evidence of the action of cytokines and growth factors in renal osteodystrophy. The development of SHPT is associated with systemic and bone inflammation, as well as with bone marrow fibrosis—effects that have been shown to be attenuated by PTx, which induces changes in markers of bone inflammation.[27] The interaction between PTH and inflammatory cytokines has effects on bone remodeling[29,30] and on muscle tissue. At 6 mo after PTx, our cohort showed a reduction in C-reactive protein and circulating levels of IL-6, together with reduced expression of the muscle cytokines IL-1β, IL-17A, and TNF-α. In a previous study, histological analysis of patients with Duchenne muscular dystrophy revealed increased muscle expression of IL-17,[31] which was found to correlate with the expression of other inflammatory cytokines and with worse functionality. Elevated muscle expression of IL-1β has been described in neuromuscular inflammatory diseases and has been associated with the development of muscle atrophy in animal models of Parkinson's disease.[32]

Reduced expression of FNDC5, the precursor of irisin, an exercise-induced myokine, has been identified in PTH (1–34)-treated myotubes, and reduced expression of the PTH receptor has been identified in irisin-treated osteoblasts.[7] In

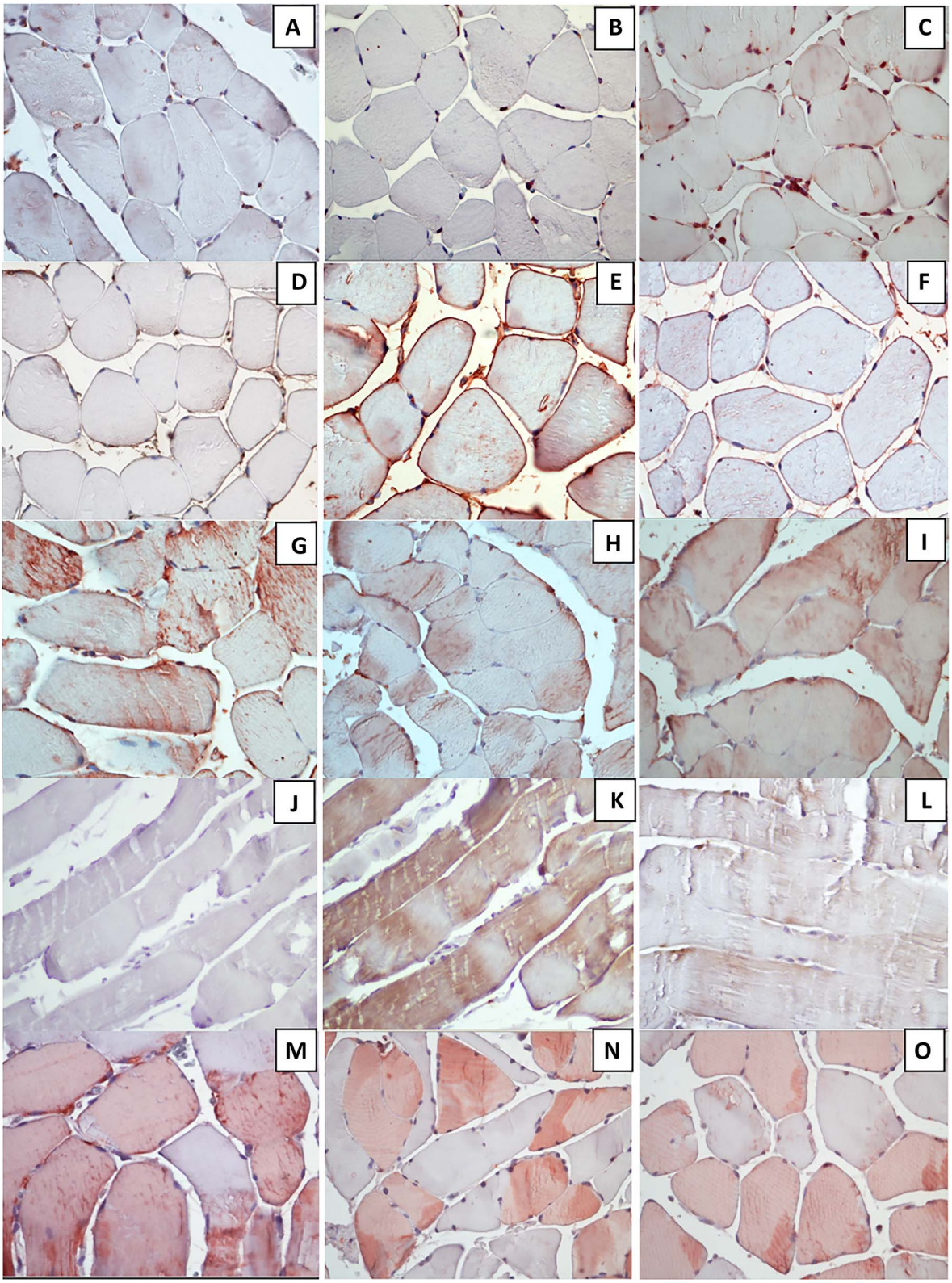


Figure 3. Immunohistochemistry in muscle biopsy samples. Protein expression of VDR (a,b,c), TGF-β (d,e,f), myostatin (g,h,i), OPN (j,k,l), and beclin-1 (m,n,o), in healthy controls (a,d,g,j,m), in patients before PTx (b,e,h,k,n) and in patients 6 mo after PTx (c,f,i,l,o). Magnification, ×400.

keeping with those findings, a significant reduction in serum irisin levels has been demonstrated in postmenopausal women with primary hyperparathyroidism.[7] Despite the absence of changes in systemic irisin in our cohort, improved muscle quality was confirmed by the post-PTx increase in the muscle concentration of irisin, which was more pronounced in patients with lower PTH levels. That indicates that better post-PTx muscle quality is associated with tighter control of PTH.

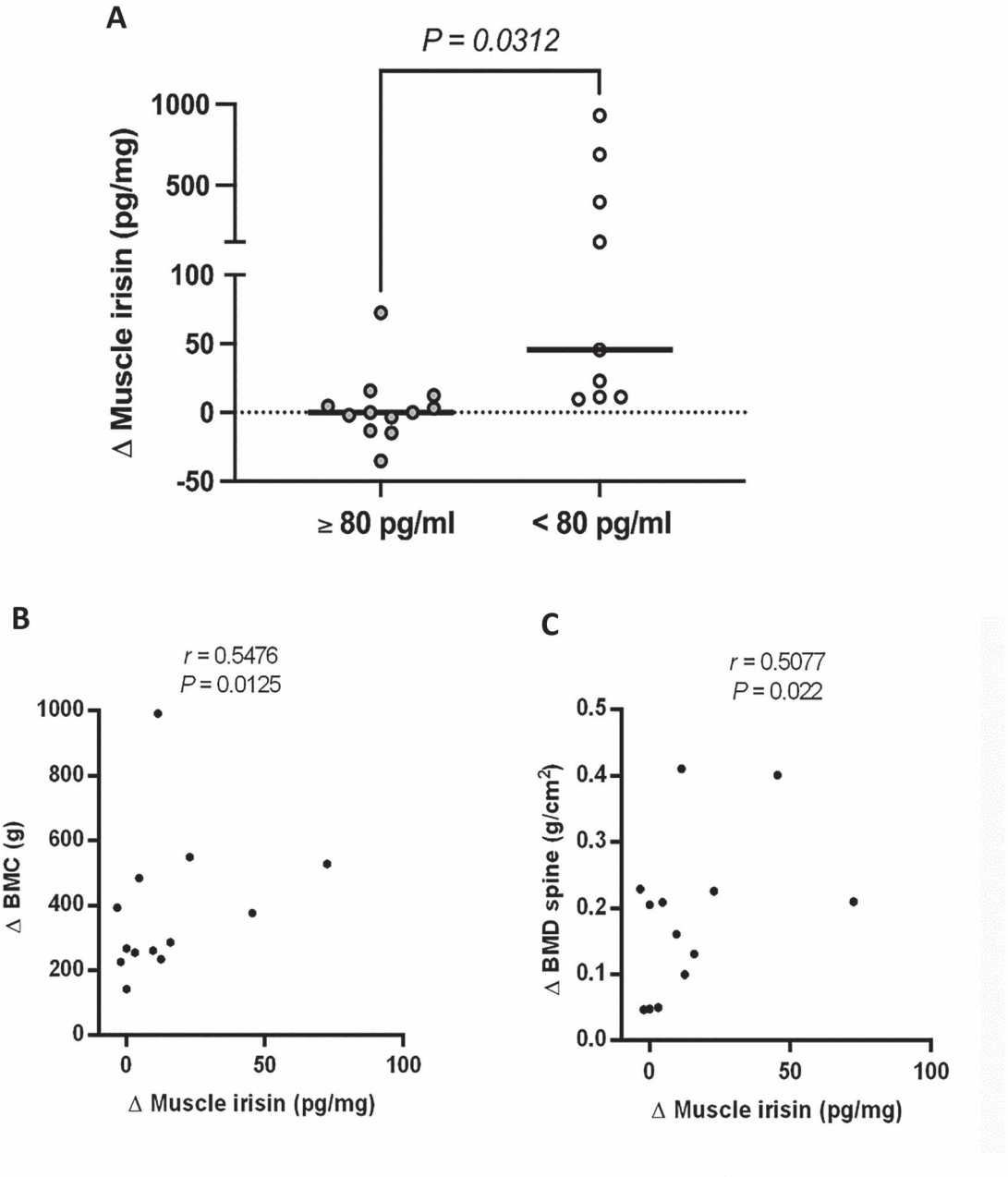


Figure 4. Changes in DXA parameters after PTx and their association with the post-PTx change in muscle irisin. (a) Variation in muscle irisin concentration according to final PTH levels. (b) Correlation between the variation in muscle irisin concentration and the variation in BMC. (c) Correlation between the variation in muscle irisin concentration and the variation in BMD in the spine.

Our tissue analysis also revealed impaired autophagic flux and changes in muscle turnover. Autophagy dysfunction is pathogenic in some congenital muscular dystrophies,[33] and beclin-1 is a key player in autophagy induction. Verzola et al.[34] analyzed muscle tissue from 22 patients with stage 5 CKD and demonstrated downregulation of *IGF-1* and *AKT*, together with upregulation of *MSTN* and *IL-6*. In our cohort, the influence of myostatin was not confirmed, although SHPT was associated with reductions in expression of *AKT* and *FOXO3*. In patients with SHPT, we noted reduced expression of the autophagy-related protein beclin-1, without changes after PTx, and reduced phosphorylated/total AKT concentration, which was attenuated after PTx. That illustrates the effect of SHPT on protein synthesis capacity and its deleterious effect on muscle autophagy.

Mitochondrial dysfunction induces a tissue stress response, leading to increased muscle expression of FGF21,[35] and the FGF21 knockout in mice has been shown to protect against fasting-induced loss of muscle mass, whereas increased FGF21-induced autophagy and muscle atrophy.[8] In our cohort, *FGF21* gene expression tended to be higher in the patients with CKD than in the controls, and protein expression of FGF21 in muscle was high and showed only a slight reduction after PTx. Therefore, even though PTx has no apparent influence on it, mitochondrial dysfunction is one of the mechanisms involved in the development of CKD myopathy.

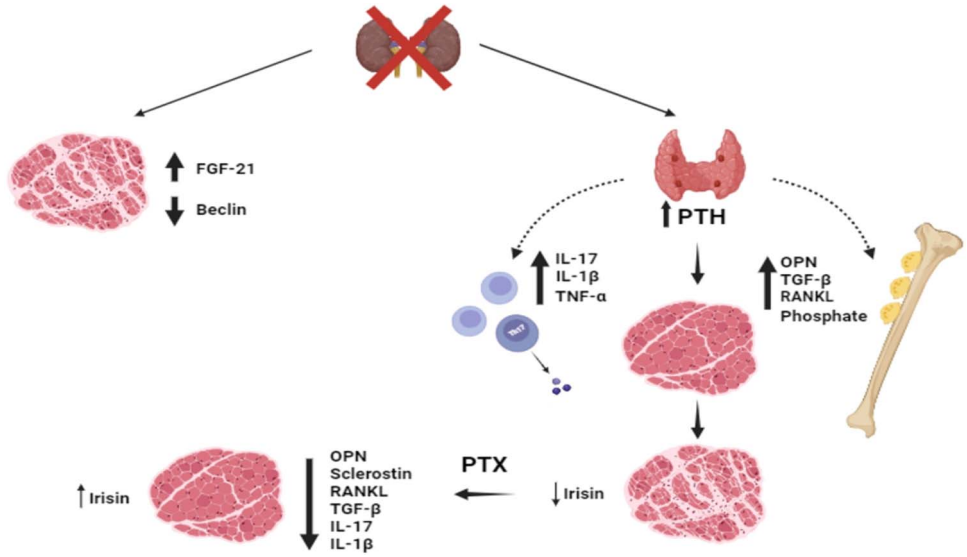


Figure 5. Proposed mechanisms of action of SHPT and PTx in muscle tissue. Created with Biorender.com

Finally, the post-PTx improvement in muscle strength might be due to adaptations in neuromuscular transmission.[36] Although there was no gain in muscle mass in our patients, PTx enhanced muscle quality, a finding that could also be attributable to the effect of phosphate or vitamin D on muscle. There is experimental and clinical evidence that phosphate overload has a negative effect on muscle strength.[37-39] In our patients, there was a significant reduction in phosphate, and that reduction could have a beneficial effect on muscle function. The direct effect of FGF23 on skeletal muscle remains unclear.[40] Although muscular FGF23 did not change significantly, the increase in number of steps negatively correlated with its variation in our patients. In addition, their 25OHD and $1,25(OH)_2D$ levels increased after supplementation during postoperative hungry bone phase. There is experimental evidence that calcitriol influences myocytes through genomic effects, involving activation of the VDR in the cell nucleus to drive differentiation and proliferation, as well as nongenomic effects related to calcium influx into cells.[41] In the present study, immunohistochemistry showed a significant increase in muscle VDR expression after PTx.

To our knowledge, this is the first prospective study evaluating the effects of severe SHPT on muscle and correlating CKD-MBD parameters with the effects of PTx on myokine levels, gene expression, and protein expression in muscle. We believe that this is also the first study to correlate CKD-MBD parameters with proteins related to muscle metabolism.

Our study has some limitations. The number of patients is relatively small, and we are aware that there are as yet no well-defined criteria for the diagnosis of sarcopenia in the CKD population. Therefore, we considered the criteria applied in other populations. In addition, as bone biopsies were not performed, it was not possible to identify the exact role of bone on the expression of specific proteins in muscle tissue or disentangle the effects of the CKD-MBD agents from those of PTH, phosphate, and FGF23. However, we evaluated proteins related to muscle metabolism not only by determining their serum levels but also by using 3 different methods to analyze them in the muscle compartment: gene expression analysis, multiplex protein quantification, and immunohistochemistry. Moreover, the comparison between changes in serum and muscular concentration of some proteins highlights local changes. Despite no changes in serum RANKL, its muscular concentration dropped significantly after PTx, as well as IL-1β, IL17A, and TNF- α concentrations. For irisin, despite no changes in its serum concentration, its muscular gene expression and protein concentration increased, and while serum sclerostin doubled, its muscular concentration decreased around 10% after PTx. Based on these findings, we hypothesize that PTx drives changes at the muscular level in patients with CKD.

Although our patients showed no post-PTx changes in muscle mass, there was a significant post-PTx improvement in muscle quality. We can speculate that PTx stopped the progression of SHPT-induced muscle wasting.

In conclusion, SHPT plays a role in CKD-related muscle dysfunction, and its effective treatment can be a strategy to improve functional performance. A unifying model to explain our findings is presented in Figure 5. Bone-specific proteins seem to influence muscle quality by regulating muscle fibrosis and inflammation, both of which improve after PTx.

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Author contributions

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(Visualization, writing—review and editing), Vanda Jorgetti (Data curation, Investigation, Visualization, Methodology, Supervision), and Rosa M.A. Moysés (Conceptualization, Data curation, Investigation, Formal analysis, Methodology, Visualization, Resources, Supervision, Project administration, Funding acquisition, Writing—review and editing)

Supplementary material

Supplementary material is available at *JBMR Plus* online.

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Conflicts of interest

C.M.A. received payment for lectures from Astra Zeneca, Fresenius Medical Care and Baxter healthcare. All other authors declared no competing interests.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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