

THE ASSOCIATION BETWEEN sRANKL/OPG SYSTEM, SERUM PTH AND VOLUMETRIC BONE MINERAL DENSITY IN GROWING RATS WITH EXPERIMENTAL CHRONIC KIDNEY DISEASE

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Introduction

The extremely important complication of kidney diseases is the chronic kidney disease-mineral and bone disorder (CKD-MBD). This syndrome refers to a constellation of 1) biochemical abnormalities in concentration of calcium, phosphorus, parathyroid hormone (PTH) or vitamin D metabolism; 2) abnormalities in bone including turnover, mineralization, volume, linear growth and strength; 3) vascular or other soft tissue calcification. The osteoprotegerin (OPG) and receptor activator of NF- κ B ligand (RANKL) play a central role in the regulation of bone turnover, but their role in the pathogenesis of renal bone disease remains unclear. The aim of our study was to investigate the role of RANKL/OPG complex in the context of CKD-MBD in growing organism.

Materials and methods

Forty-four, 4 weeks old Wistar male rats were divided into two groups: with chronic kidney disease induced by surgical 5/6 subtotal nephrectomy (CKD, n=22), and sham-operated (CON, n=22). After one (CON-1; CKD-1) and three-month (CON-3; CKD-3, n = 11 per each group; respectively) of the surgery rats were subjected to analysis. We evaluated the concentration of OPG and soluble RANKL (sRANKL) in serum and homogenates from trabecular and cortical femur tissue of growing rats with surgically-induced mild CKD. Additionally, we analyzed the possible association between sRANKL/OPG system and PTH, femur weight and volumetric BMD (vBMD).

Statistical analysis

Shapiro-Wilk's test of normality was used for data distribution analysis. The normally distributed data were expressed as mean \pm SD or median (full range). The data were analyzed using a two-way analysis of variance (ANOVA). The two independent factors were: group (CKD or control) and age (1 or 3 months). If the ANOVA results showed significant differences ($p < 0.05$), post hoc Bonferroni test was used to test the level of significance between individual groups. The correlations between study variables were calculated by Spearman's rank correlation analysis. A two-tailed p value < 0.05 was considered statistically significant.

Results

Correlations between OPG/RANKL system and serum PTH

In CKD animals, we observed the opposite correlations between bone OPG, sRANKL levels and serum PTH concentrations. The positive relationships were between bone OPG and PTH ($R=0.564$, $p=0.014$; $R=0.228$, NS). In contrast, the inverse associations were noted between PTH and sRANKL ($R=-0.536$, $p=0.012$; $R=-0.477$, $p=0.029$) and sRANKL/OPG ratios ($R=-0.639$, $p=0.004$; $R=-0.509$, $p=0.026$) in both analyzed bone regions.

Table 1. Biochemical parameters in controls and chronic kidney disease (CKD) rats after one and three months of disease progression.

	CON-1,	CKD-1	CON-3	CKD-3
Creatinine, μmol/l	27.40 \pm 2.65	38.90 \pm 6.19 **	34.45 \pm 3.54	53.04 \pm 4.42 *** ###
BUN, mmol/l	8.39 \pm 1.17	13.57 \pm 2.41 **	6.66 \pm 0.43	11.89 \pm 1.68 ***
PTH, pg/ml	256 (84-612)	300 (130-525)	320 (185-590)	490 (308-1338) *#

BUN=blood urea nitrogen, PTH=parathyroid hormone
Data are mean \pm SD or median (full range). * $p < 0.05$, ** $p < 0.01$, controls versus appropriate CKD group; # $p < 0.05$, ### $p < 0.001$ CKD 1 month versus CKD 3 months

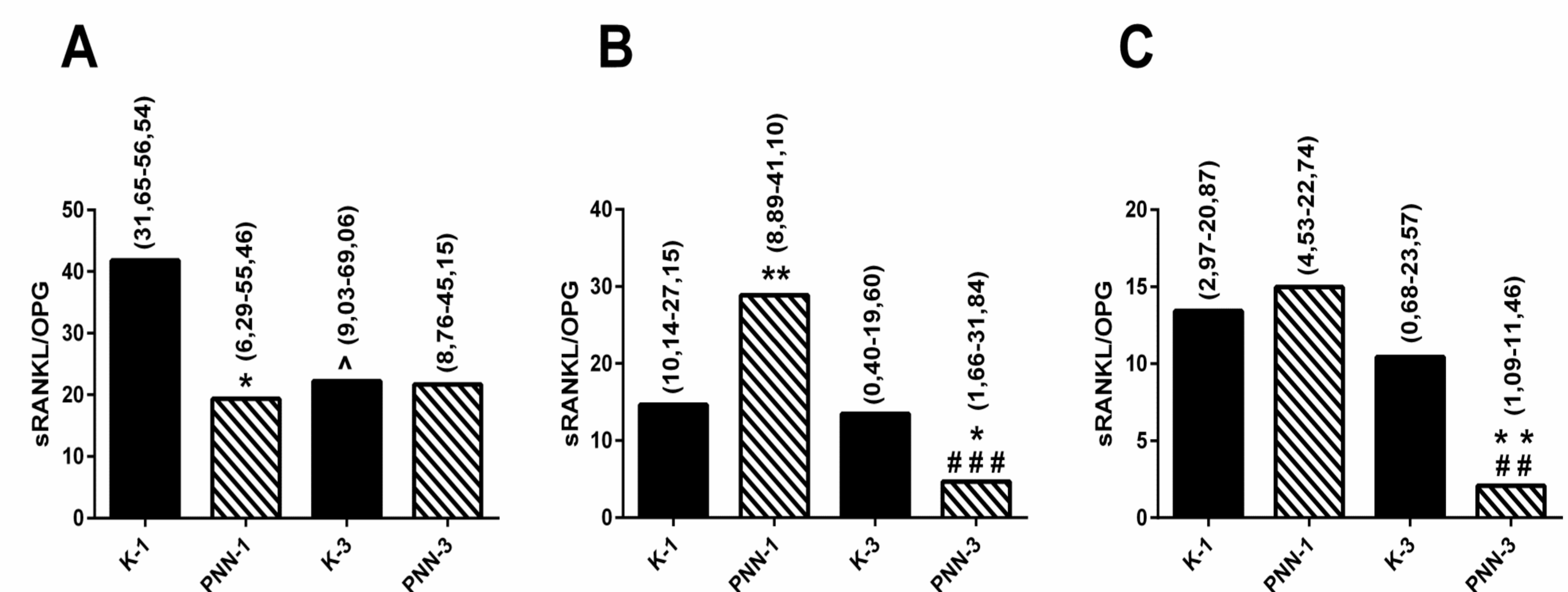


Fig.1 The changes in serum (A), trabecular (B) and cortical (C) levels of sRANKL/OPG ratio in controls and appropriate chronic kidney disease (CKD) rats.

Data are presented as median (full range), n = 11 in each group. * $p < 0.05$, ** $p < 0.01$, controls versus appropriate CKD group; ^ $p < 0.05$ CON-1 versus CON-3; ### $p < 0.001$ CKD-1 versus CKD-3

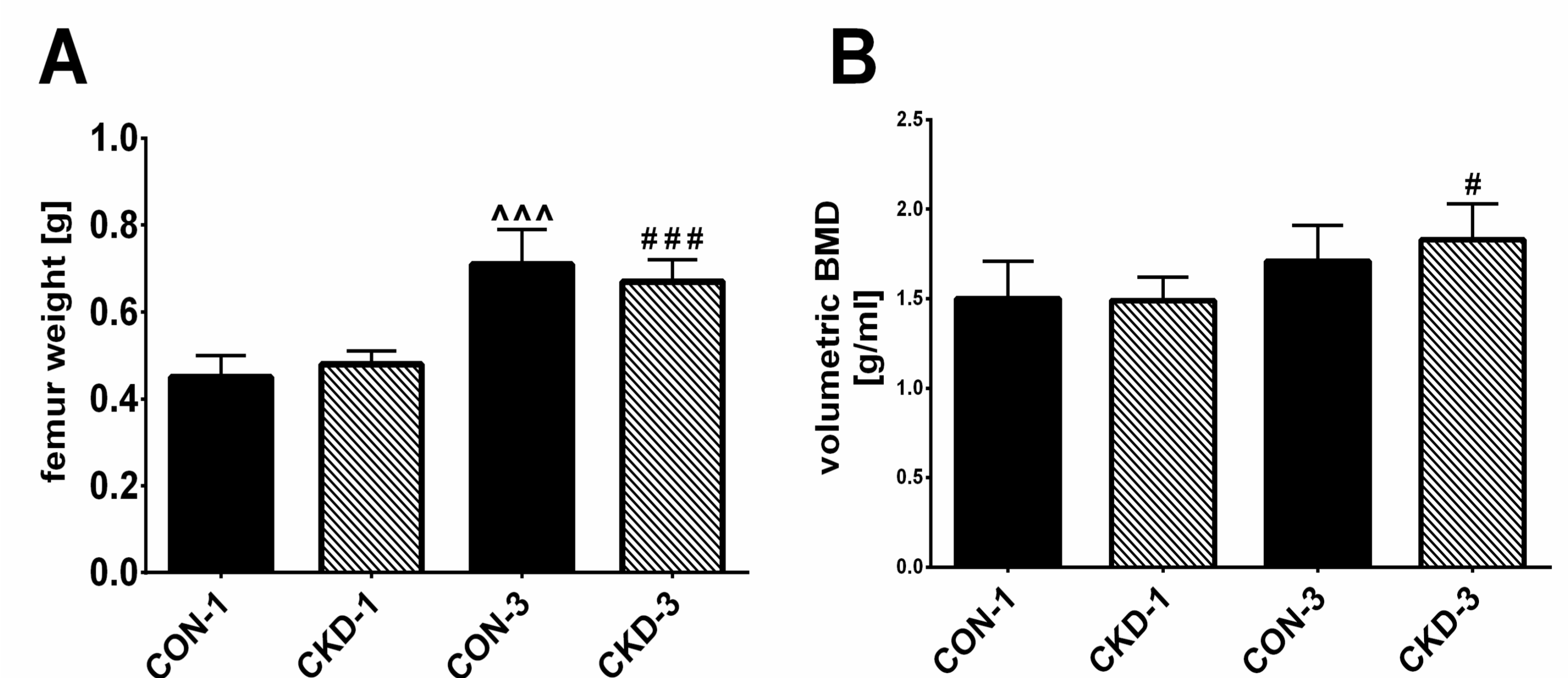


Fig.2. Femur weight (panel A) and volumetric bone mass density (BMD) (panel B) of controls (CON) and chronic kidney disease (CKD) rats.

Data are presented as mean \pm SD. ^^^ $p < 0.001$ CON-1 versus CON-3; # $p < 0.05$, ### $p < 0.001$ CKD-1 versus CKD-3

Table 2. The association between sRANKL/OPG system in trabecular and cortical bone tissue, PTH in serum and femoral weight and volumetric bone mass density (vBMD) in rats with chronic kidney disease (CKD).

	Femoral Weight	vBMD
Trabecular OPG	R = 0.544 p = 0.016	R = 0.216 NS
Trabecular sRANKL	R = -0.669 p = 0.0007	R = -0.745 p < 0.0001
Trabecular sRANKL/OPG	R = -0.689 p = 0.001	R = -0.339 NS
Cortical sRANKL	R = -0.477 p = 0.025	R = -0.229 NS
Cortical sRANKL/OPG	R = -0.574 p = 0.008	R = -0.472 p = 0.036
Serum PTH	R = 0.500 p = 0.021	R = 0.565 p = 0.008

Conclusions

1. The impaired renal function affects the level of OPG, sRANKL and PTH in serum and bone tissues.
2. PTH exerts different effect on sRANKL/OPG system in bone tissues.
3. The observed PTH-mediated increase of OPG in the course of CKD could represent a compensatory mechanism to the negative bone balance induced by uremia in order to maintain an adequate bone mass in animals with CKD.

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