

LOWER BONE TURNOVER AND SKELETAL PTH RESPONSIVENESS IN JAPANESE COMPARED TO EUROPEAN PATIENTS ON HEMODIALYSIS

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Abstract

Context: Parathyroid hormone (PTH) treatment targets for patients receiving hemodialysis (HD) are lower in Japan than in Europe. Whether this translates to lower bone turnover is unknown and could depend on skeletal PTH responsiveness.

Objective: This study investigates whether skeletal PTH responsiveness is better preserved in Japanese vs European patients receiving HD.

Methods: This is a post hoc analysis of data from 2 prospective cohort studies, using a case-control design. Patients receiving chronic intermittent HD therapy were eligible for inclusion. Participating Belgian and Japanese patients (n = 374) were matched 1:1 by age (59 ± 12 years), sex (66% male), diabetes (34%), and dialysis duration (39 months [22–63 months]). PTH, bone-specific alkaline phosphatase (BALP), and tartrate-resistant acid phosphatase isoform 5b (TRAP5b) were measured centrally in Liège, Belgium.

Results: Japanese patients had lower levels of iPTH (207 vs 268 pg/mL; $P < .001$), BALP (15.3 vs 24.5 µg/L; $P < .001$), and TRAP5b (3.35 vs 5.79 U/L; $P < .001$). Linear regression analyses revealed lower levels of bone turnover markers for any given level of PTH in Japanese vs Belgian patients, indicating lower skeletal PTH responsiveness. Consistently, bone turnover markers were significantly lower in

Japanese vs Belgian patients when stratifying or matching according to PTH levels. Male sex, obesity, and hyperphosphatemia were the main determinants of the bone turnover marker/PTH ratios.

Conclusion: Japanese patients receiving HD have lower bone turnover than their European counterparts, even at similar PTH levels. The rationale for the current regional differences in PTH treatment targets remains obscure and deserves further attention.

Abbreviations: ALP, alkaline phosphatase; BALP, bone-specific alkaline phosphatase; BMI, body mass index; CKD, chronic kidney disease; HD, hemodialysis; iPTH, intact parathyroid hormone; PTH, parathyroid hormone; TRAP5b, tartrate-resistant acid phosphatase isoform 5b; UNL, upper limit of normal; wPTH, whole parathyroid hormone.

Secondary hyperparathyroidism is a universal complication in chronic kidney disease (CKD) (1). Elevated levels of parathyroid hormone (PTH) may represent a compensatory mechanism to maintain mineral homeostasis in early stages of CKD (2), but in later stages, hyperparathyroidism becomes maladaptive with steadily increasing levels of PTH conferring negative effects on the skeletal, metabolic, immunological, and cardiovascular systems (3). Both high and low levels of PTH are associated with poor outcomes related to CKD mineral and bone disorder, including cardiovascular events (4, 5), fractures (6, 7), and all-cause mortality (8, 9). The optimal PTH target range for bone and vascular health in CKD is not known.

Marked regional and ethnical differences exist with regard to PTH control, with the highest levels seen in US African American individuals and the lowest in Japanese patients on hemodialysis (HD) (10). These differences largely reflect a lack of consensus on PTH treatment targets. While the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines followed in Europe and the United States suggest that PTH should be kept within 2 to 9 times the upper limit of normal (UNL) (11), the Japanese Society for Dialysis Therapy recommends a lower and more narrow PTH target of 60 to 240 pg/mL, or approximately 1 to 4 times the UNL (12).

A possible explanation for these disparities could be population differences in the responsiveness to PTH in target organs, such as the skeleton. Epidemiological studies investigating the association between PTH levels and survival in patients on HD show a shift toward a lower PTH optimum in Japanese (13, 14) compared to European or American patients (9). Ethnic differences in the skeletal response to PTH have been demonstrated, as US African American patients have lower bone remodeling rates at comparable PTH levels compared to US White patients with CKD (15). Information on skeletal PTH responsiveness in other ethnicities, or between different regions of the world, is lacking.

While the gold standard to assess PTH responsiveness is by change in serum calcium after infusion of recombinant (1-34) PTH, the ratio of circulating bone turnover marker over PTH levels has been suggested as a proxy of skeletal PTH responsiveness (16). Bone turnover markers are fragments of collagen, or enzymes originating from bone cells, that are released into the systemic circulation during skeletal remodeling (17). As such, circulating levels are considered a measure of the current whole-skeletal bone turnover.

In this study, we tested the hypothesis that skeletal PTH responsiveness is better preserved in Japanese patients on HD; in which case, bone turnover markers could be comparable, despite the well-known differences in PTH levels. We assessed parameters of mineral metabolism, circulating bone turnover markers, and the ratio of bone turnover markers by PTH in a matched cohort of Belgian and Japanese HD patients, with key biomarkers analyzed in a central laboratory.

Materials and Methods

STUDY DESIGN AND COHORTS

Patients receiving chronic intermittent HD therapy participating in 1 of 2 observational, prospective cohort studies were included in this analysis. The Tokai Dialysis Prospective Cohort Study included patients receiving chronic intermittent HD at 10 dialysis facilities across Japan. Patients were eligible for inclusion if they were older than 20 years and had received HD thrice weekly for more than 3 months as of June 2012 (18). The second cohort consisted of adult patients (age > 18 years) referred for single kidney transplantation at the University Hospitals Leuven in Belgium, participating in studies investigating bone health (19). For the present analysis, only patients receiving HD therapy were eligible for participation. In both cohorts, bisphosphonate users were excluded (Japanese cohort, $n = 5$ [0.8%]; Belgian cohort, $n = 8$ [2%]), as were patients with PTH levels at the extremes, defined as either below the lower normal limit (Japanese cohort, $n = 46$ [7%]; Belgian cohort, $n = 3$ [0.8%]), or above 15 times the UNL (Japanese cohort, $n = 2$ [0.3%]; Belgian cohort, $n = 15$ [4%]) of the assay. Patients who had undergone a parathyroidectomy were included if their PTH levels were within these limits. Propensity score modeling was used to match for age, sex, diabetes, and dialysis duration, as detailed later.

BIOCHEMICAL ANALYSES

In the Tokai cohort, blood sampling was performed immediately before a HD session, while in Leuven, sampling was random with respect to last HD session, as blood tests were drawn at the time of admission for kidney transplantation. For both cohorts, blood samples were drawn in a nonfasting state. Creatinine, albumin, hemoglobin, phosphate, and total calcium were measured using standard laboratory techniques at each center. Total calcium levels were corrected for albumin levels by the Payne formula (20). Total alkaline phosphatase (ALP) levels were normalized to the assay UNL, which was 359 U/L for the Tokai cohort, and 113 U/L for men and 100 U/L for women in the Leuven cohort.

Stored blood samples were shipped to the Department of Clinical Chemistry at University Hospital Liège (Liège, Belgium) for centralized measurement of PTH and bone turnover markers. Serum samples had been stored at -80°C until analysis. Whole (wPTH) and intact (iPTH) PTH were measured by electrochemiluminescence immunoassays (Cobas Elecsys, Roche Diagnostics). The normal reference ranges were 4.0 to 49.0 pg/mL for wPTH (21) and 13.7 to 50.2 pg/mL for iPTH (22). Bone-specific alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase isoform 5b (TRAP5b) were measured using the IDS-iSYS instrument (Immuno Diagnostic Systems). The assay-specific reference

values according to the manufacturer were: BALP 5.7 to 32.9 $\mu\text{g/L}$ for men and 5.5 to 27.1 $\mu\text{g/L}$ for postmenopausal women and TRAP5b 1.4 to 6.1 U/L for men and 1.1 to 6.9 U/L for postmenopausal women.

STATISTICAL ANALYSIS

Data are given as mean $\pm$ SD, median (interquartile range, IQR), or n (%), as appropriate, with between-group comparisons by *t* test, Wilcoxon rank sum test, or Pearson χ^2 test, respectively. We developed a propensity score for the predictor “Center” (Tokai vs Leuven) using a multivariable logistic regression model including age, sex, diabetes, and dialysis duration, calculating the log of the odds of the propensity score. Model optimization indicated nonlinearity between age and duration and the log odds, and appropriate terms were therefore added to the logistic regression. Subsequent model goodness of fit was acceptable (Hosmer-Lemeshow *P* = .27). A one-to-one greedy match was performed, using a caliper of 0.025, yielding 187 matched pairs. Balance between covariates was assessed by standardized differences (all < 0.1 SD). We used multivariable linear regression models to investigate determinants of skeletal responsiveness, including potential confounders and all variables differing significantly across tertiles of bone turnover markers/iPTH ratios. Finally, to match the 2 cohorts for PTH levels, we randomly selected 1 patient from each center within iPTH categories of 5 pg/mL (if iPTH < 275 pg/mL), 10 pg/mL (if iPTH > 275 pg/mL), or 25 pg/mL (if iPTH > 400 pg/mL), achieving 63 pairs. Statistical analyses were performed using the commercially available software STATA IC version 16.1 (StataCorp LP).

Results

Demographic data for the 2 cohorts are shown in Table 1. Belgian patients were taller, heavier, and more obese than their Japanese counterparts. Belgian patients were also more likely to have coronary artery disease and to have suffered a previous fracture. Biochemical measurements revealed the expected differences in mineral metabolism, with lower levels of PTH and higher levels of phosphate in Japanese compared to Belgian patients. Japanese patients were more likely to receive PTH-lowering therapy, particularly active vitamin D, either oral or intravenous.

BONE TURNOVER

Biochemical markers of skeletal remodeling were significantly lower in Japanese as compared to Belgian patients (see Table 1). Using the assay-specific reference intervals of the bone turnover markers, the proportion of Japanese patients with levels within the normal range was 88% for BALP and 86% for TRAP5b, whereas the corresponding proportions in the Belgian cohort were 67% and 57%, respectively (Fig. 1).

Patients were categorized as having low, normal, or high bone turnover by applying diagnostic cutoffs of BALP and TRAP5b as previously determined (23), with low turnover defined as both BALP less than 24.2 $\mu\text{g/L}$ and TRAP5b less than 3.44 U/L, and high turnover as both BALP greater than 33.7 $\mu\text{g/L}$ and

TRAP5b greater than 5.05 U/L. By these cutoffs, the prevalence of high bone turnover was 29% in the Belgian and 5% in the Japanese cohort ($P < .001$) (Fig. 2).

Table 1. Demographic and biochemical data for participating Belgian and Japanese patients

	Belgian cohort (n = 187)	Japanese cohort (n = 187)	P
Demographic variables			
Age, y	59.05 ± 11.62	58.60 ± 11.71	.71
Male sex	123 (66%)	125 (67%)	.83
Diabetes mellitus, any type	62 (33%)	66 (35%)	.66
Dialysis duration, mo	39 (23-64)	38 (19-59)	.57
Height, cm	168.8 ± 9.1	163.0 ± 10.3	< .001
Weight, kg	72.4 ± 15.2	59.9 ± 15.9	< .001
Body mass index	25.3 ± 4.4	22.3 ± 4.4	< .001
Systolic blood pressure, mm Hg	141 ± 21	144 ± 21	.24
Diastolic blood pressure, mm Hg	77 ± 12	76 ± 13	.54
Cardiovascular disease	45 (24%)	27 (14%)	.02
Coronary artery disease	30 (16%)	16 (9%)	.03
Cerebrovascular disease	15 (8%)	13 (7%)	.69
Peripheral artery disease	9 (5%)	3 (2%)	.08
Previous fracture	43 (23%)	13 (7%)	< .001
Previous parathyroidectomy	19 (10%)	3 (2%)	< .001
Calcimimetic use	24 (13%)	37 (20%)	.07
Active vitamin D use	78 (42%)	128 (68%)	< .001
Biochemistry			
Albumin, g/L	4.25 ± 0.61	3.80 ± 0.33	< .001
Hemoglobin, g/dL	11.91 ± 1.59	10.27 ± 0.99	< .001
Total calcium, mg/dL	9.13 ± 0.73	8.96 ± 0.79	.03
Calcium corrected, mg/dL	8.88 ± 0.86	9.21 ± 0.72	< .001
Phosphate, mg/dL	4.28 ± 1.27	4.94 ± 1.05	< .001
iPTH, pg/mL	268 (152-440)	166 (87-264)	< .001
wPTH, pg/mL	161 (97-261)	110 (60-170)	< .001
Total ALP, xUNL	0.83 (0.64-1.12)	0.57 (0.45-0.74)	< .001
BALP, µg/L	24.5 (16.4-35.1)	15.3 (11.7-22.2)	< .001
TRAP5b, U/L	5.79 (4.01-7.80)	3.35 (2.33-4.74)	< .001
ALP/iPTH ratio	0.16 (0.10-0.31)	0.20 (0.11-0.31)	.26
BALP/iPTH ratio	0.098 (0.061-0.177)	0.106 (0.066-0.189)	.48
TRAP5b/iPTH ratio	0.022 (0.013-0.040)	0.022 (0.014-0.037)	.99

Data are mean ± SD, median (interquartile range), or n (%) with P by t test, Wilcoxon rank sum test, or Pearson χ^2 test, respectively. Abbreviations: ALP, alkaline phosphatase; BALP, bone-specific alkaline phosphatase; iPTH, intact parathyroid hormone; TRAP5b, tartrate-resistant acid phosphatase isoform 5b; wPTH, whole parathyroid hormone; xUNL, times upper normal limit of the assay.

Figure 1. Proportion of Belgian and Japanese patients with circulating levels of bone-specific alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase isoform 5b (TRAP5b) within the assay-specific reference range.

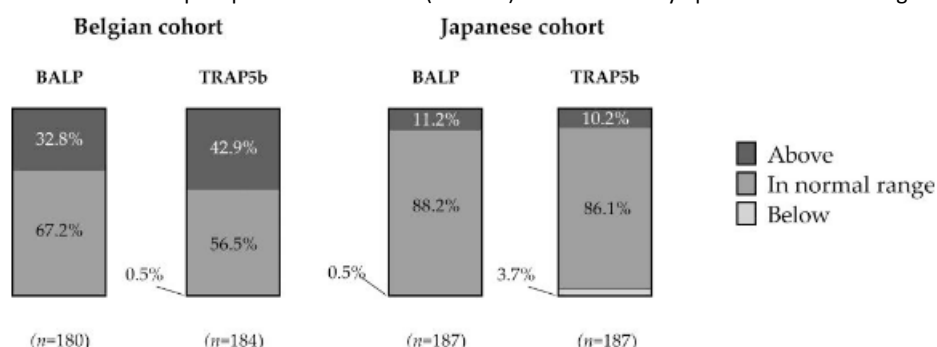
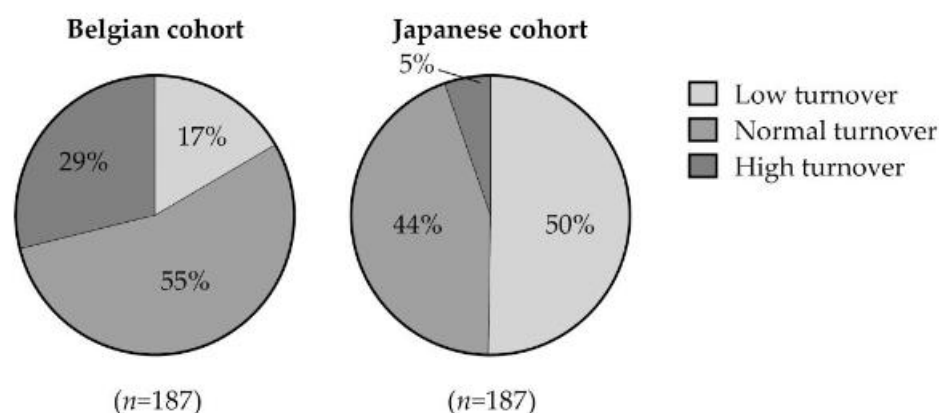


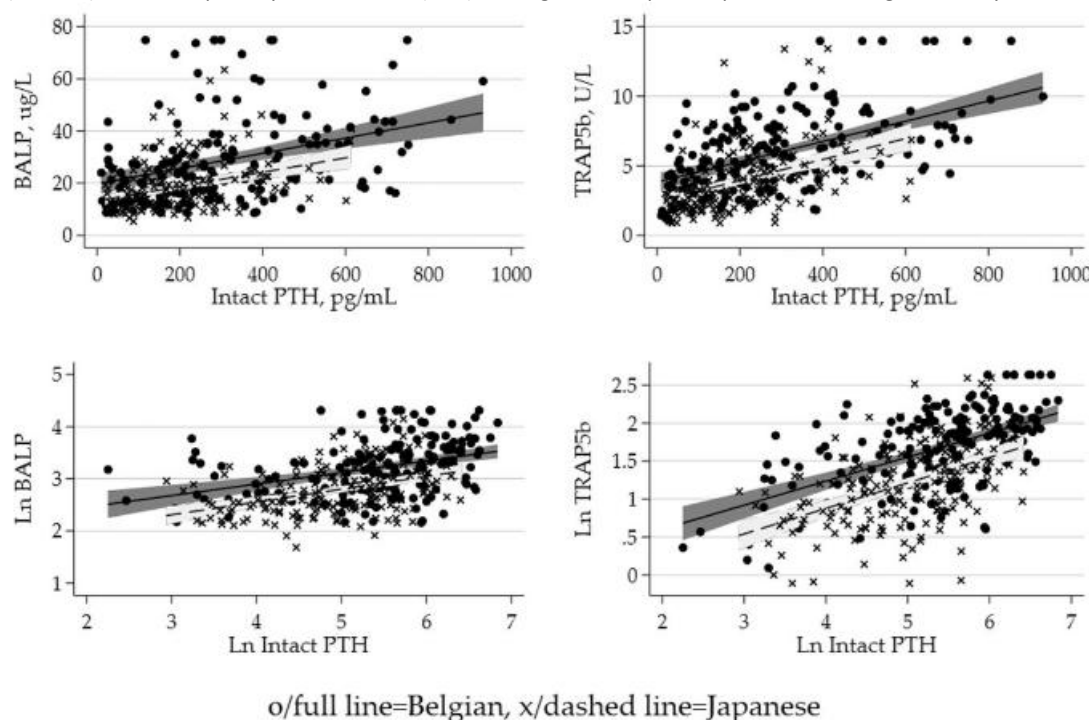
Figure 2. Bone turnover classification by the combination of bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase isoform 5b in Belgian and Japanese patients receiving hemodialysis.



PARATHYROID HORMONE RESPONSIVENESS

Scatterplots of the circulating levels of bone turnover markers over iPTH revealed systematically lower levels of BALP and TRAP5b in Japanese compared to Belgian patients across the range of iPTH (Fig. 3). By linear regression, Belgian patients had 6.9 ± 1.4 $\mu\text{g/L}$ higher BALP and 1.34 ± 0.25 U/L higher TRAP5b at any given level of iPTH ($P < .001$, both). Consistently, when dividing patients into quartiles of iPTH, circulating levels of BALP and TRAP5b were significantly lower in Japanese vs Belgian patients for each iPTH category (Supplementary Fig. 1) (24). Results were comparable for total ALP over iPTH, with both expressed as $\times\text{UNL}$ of the assay (Supplementary Fig. 2) (24).

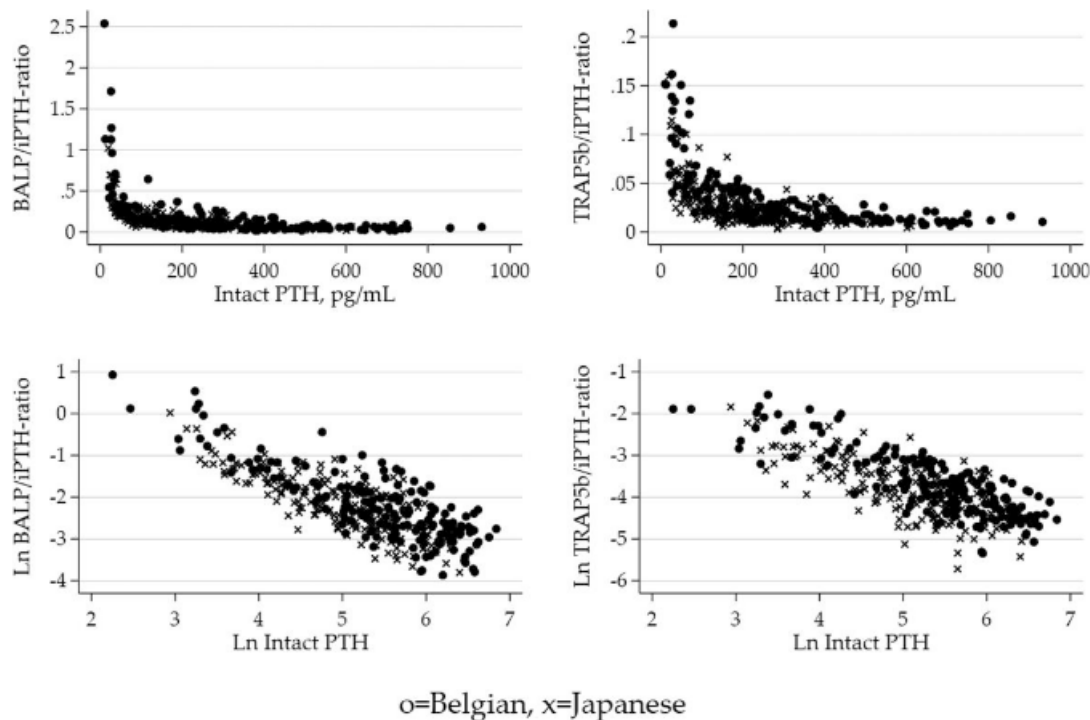
Figure 3. Relationship between bone-specific alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase isoform 5b (TRAP5b) and intact parathyroid hormone (PTH), in Belgian and Japanese patients receiving hemodialysis.



DETERMINANTS OF SKELETAL PARATHYROID HORMONE RESPONSIVENESS

The ratios of circulating bone turnover marker over iPTH levels were used as surrogate measures of skeletal PTH responsiveness. There was an inverse relationship between the bone turnover marker/iPTH ratios and iPTH levels; ratios were highest at low-normal iPTH and decreased rapidly with increasing iPTH levels (Fig. 4).

Figure 4. The relationship between intact parathyroid hormone (iPTH) levels and the bone turnover marker/iPTH-ratios. BALP, bone-specific alkaline phosphatase; TRAP5b, tartrate-resistant acid phosphatase isoform 5b.



To identify determinants of skeletal PTH responsiveness, we divided patients into tertiles of bone turnover marker/iPTH ratios. In Japanese and Belgian patients, a low TRAP5b/iPTH ratio was associated with male sex, obesity, hyperphosphatemia, and hyperparathyroidism (Table 2). Results were similar for tertiles of the BALP/iPTH ratio (Supplementary Table 1) (24).

Next, we performed multivariable linear regression analyses, including potential confounders and all variables significantly associated with the bone turnover marker/iPTH ratios. Male sex, obesity, and hyperphosphatemia were consistent determinants of a lower bone turnover marker/iPTH ratio (Table 3).

Lastly, as hyperparathyroidism was a strong, negative determinant of skeletal PTH response, we further matched the 2 cohorts on iPTH levels, resulting in 63 patient pairs. After matching, levels of ALP, BALP, and TRAP5b, as well as ratios of bone turnover markers over iPTH, were significantly lower in the Japanese compared to Belgian patients (Table 4).

Table 2. Demographic and biochemical variables across tertiles of the TRAP5b/iPTH-ratio in participating Belgian and Japanese patients

	Belgian cohort			Japanese cohort		
	Lowest (n = 60)	Middle (n = 61)	Highest (n = 62)	Lowest (n = 61)	Middle (n = 63)	Highest (n = 63)
TRAP5b/iPTH ratio	0.011 (0.010-0.013)	0.021 (0.018-0.025)	0.047 (0.039-0.087) ^a	0.011 (0.009-0.014)	0.022 (0.018-0.026)	0.044 (0.037-0.061) ^a
Age, y	60 ± 10	61 ± 10	56 ± 14 ^a	56 ± 11	60 ± 12	60 ± 12 ^b
Male sex, %	48 (80.0%)	37 (60.7%)	36 (58.1%) ^a	48 (78.7%)	41 (65.1%)	36 (57.1%) ^a
Diabetes, %	19 (31.7%)	22 (36.1%)	20 (32.3%)	19 (31.1%)	27 (42.9%)	20 (31.7%)
Dialysis duration, mo	32 (24-54)	44 (21-67)	44 (23-68)	44 (22-65)	37 (20-59)	33 (16-57)
BMI	27.1 ± 4.7	24.9 ± 3.7	24.0 ± 4.3 ^a	24.5 ± 5.0	21.8 ± 4.1	20.8 ± 3.2 ^a
Calcimimetic use	12 (20.0%)	6 (9.8%)	6 (9.7%)	13 (21.3%)	11 (17.5%)	13 (20.6%)
Active vitamin D use	30 (50.0%)	22 (36.1%)	25 (40.3%)	43 (70.5%)	45 (71.4%)	40 (63.5%)
Albumin, g/L	4.25 ± 0.51	4.41 ± 0.52	4.17 ± 0.57 ^a	3.88 ± 0.31	3.76 ± 0.31	3.74 ± 0.36 ^a
Hemoglobin, g/dL	11.78 ± 1.51	12.09 ± 1.58	11.82 ± 1.71	10.43 ± 0.85	10.26 ± 1.22	10.13 ± 0.85
Total calcium, mg/dL	9.07 ± 0.83	9.26 ± 0.68	9.07 ± 0.69	9.01 ± 0.75	8.76 ± 0.67	9.12 ± 0.90 ^a
Calcium corrected, mg/dL	8.83 ± 0.77	8.85 ± 0.83	8.91 ± 0.92	9.19 (0.69)	9.04 (0.63)	9.40 (0.79) ^a
Phosphate, mg/dL	4.69 ± 1.29	3.98 ± 1.25	3.90 ± 1.23 ^a	5.06 ± 1.07	4.94 ± 1.04	4.82 ± 1.04
wPTH, pg/mL	262 (206-353)	169 (119-234)	77 (39-128) ^a	168 (128-225)	113 (72-170)	55 (30-86) ^a
iPTH, pg/mL	479 (348-609)	280 (197-416)	114 (50-193) ^a	264 (208-378)	167 (113-240)	73 (42-116) ^a
Total ALP, ×UNL	0.83 (0.65-0.95)	0.93 (0.65-1.35)	0.82 (0.63-1.11)	0.56 (0.45-0.72)	0.60 (0.45-0.80)	0.54 (0.45-0.73)
BALP, µg/L	21.9 (16.4-33.6)	27.9 (17.7-44.5)	24.2 (15.0-32.1) ^b	14.3 (11.0-19.3)	17.7 (11.8-26.6)	14.9 (12.2-22.2)
TRAP5b, U/L	5.29 (3.56-6.89)	6.15 (4.07-8.85)	6.04 (3.85-8.06)	3.13 (2.02-3.91)	4.16 (2.34-5.28)	3.37 (2.45-5.26) ^a

Data are mean ± SD, median (interquartile range), or n (%) with ^a = $P < .05$ and ^b = $P < .10$ by one-way analysis of variance, Kruskal-Wallis equality-of-populations rank test, or Pearson χ^2 test, respectively. Abbreviations: ALP, alkaline phosphatase; BALP, bone-specific alkaline phosphatase; BMI, body mass index; iPTH, intact parathyroid hormone; TRAP5b, tartrate-resistant acid phosphatase isoform 5b; wPTH, whole parathyroid hormone; ×UNL, times upper normal limit of the assay.

Table 3. Determinants of skeletal parathyroid hormone responsiveness in Belgian and Japanese patients receiving hemodialysis therapy

	Ln (TRAP5b/iPTH) ratio			Ln (BALP/iPTH) ratio			Ln (ALP/iPTH) ratio		
	Model-adjusted R ² 11%			Model-adjusted R ² 10%			Model-adjusted R ² 6%		
	β	SE	P	β	SE	P	β	SE	P
Japanese nationality	-0.170	0.091	.06	-0.014	0.102	.89	0.024	0.106	.82
Age, y	0.000	0.003	.88	-0.001	0.004	.75	-0.004	0.004	.25
Male sex	-0.196	0.080	.01	-0.243	0.089	.007	-0.206	0.094	.03
BMI	-0.043	0.009	< .001	-0.027	0.010	.008	-0.129	0.010	.77
Dialysis duration, mo	0.002	0.001	.142	0.002	0.001	.11	0.000	0.001	.26
Diabetes mellitus	0.136	0.080	.09	0.151	0.089	.09	0.106	0.095	.22
Albumin, g/dL	-0.128	0.084	.131	-0.108	0.095	.26	-0.198	0.091	.03
Phosphate, mg/dL	-0.083	0.031	.008	-0.141	0.035	< .001	-0.101	0.037	.007
Calcium, mg/dL	0.039	0.050	.43	0.002	0.056	.975	0.059	0.059	.32
Active vitamin D use	-0.041	0.076	.59	-0.030	0.085	.725	-0.082	0.090	.36
Calcimimetic use	-0.004	0.102	.97	-0.146	0.114	.201	-0.209	0.121	.08

Data are linear regression β values, with SE and P values. Bold values indicate $P < .05$. Abbreviations: ALP, alkaline phosphatase; BALP, bone-specific alkaline phosphatase; BMI, body mass index; iPTH, intact parathyroid hormone; TRAP5b, tartrate-resistant acid phosphatase isoform 5b.

Table 4. Biochemical measurement of mineral metabolism in Japanese and Belgian patients, matched for levels of intact parathyroid hormone

	Belgian cohort (n = 63)	Japanese cohort (n = 63)	P
Age, y	58.78 ± 12.52	58.75 ± 11.64	.99
Male sex	41 (65.1%)	43 (68.3%)	.71
Diabetes mellitus	20 (31.7%)	20 (31.7%)	.99
Dialysis duration, mo	47.8 (24.0-64.1)	46.3 (24.0-67.0)	.84
iPTH, pg/mL	257.5 (157.2-356.1)	258.7 (156.4-355.9)	.97
wPTH, pg/mL	157.1 (110.5-217.3)	161.2 (108.7-224.0)	.97
Total calcium, mg/dL	8.96 ± 0.75	8.98 ± 0.84	.88
Calcium corrected, mg/dL	8.69 ± 0.88	9.24 ± 0.74	< .001
Phosphate, mg/dL	4.35 ± 1.25	5.02 ± 1.20	.003
Total ALP, ×UNL	0.80 (0.64-1.08)	0.57 (0.47-0.75)	< .001
BALP, µg/L	23.5 (16.5-33.3)	18.1 (13.5-25.4)	.01
TRAP5b, U/L	4.80 (3.65-7.49)	3.92 (2.67-5.28)	< .001
ALP/iPTH ratio	0.17 (0.11-0.26)	0.13 (0.08-0.19)	.004
BALP/iPTH ratio	0.09 (0.07-0.16)	0.07 (0.05-0.14)	.04
TRAP5b/iPTH ratio	0.021 (0.014-0.033)	0.015 (0.011-0.029)	.02

Data are mean ± SD or median (interquartile range) with *P* by *t* test or Wilcoxon rank sum test, respectively.
 Abbreviations: ALP, alkaline phosphatase; BALP, bone-specific alkaline phosphatase; BMI, body mass index; iPTH, intact parathyroid hormone; TRAP5b, tartrate-resistant acid phosphatase isoform 5b; ×UNL, times upper normal limit of the assay; wPTH, whole parathyroid hormone.

SENSITIVITY ANALYSES

In a sensitivity analysis excluding patients with a previous parathyroidectomy, we found no substantial differences in the relationship between bone turnover marker and PTH levels (data not shown). However, in the adjusted model for determinants of the bone turnover marker/PTH ratios, diabetes emerged as a significant determinant (Supplementary Table 2) (24). Stratifying by active vitamin D therapy similarly did not materially alter the results, and particularly, levels of bone turnover markers remained lower for any given level of PTH in Japanese vs Belgian patients not receiving active vitamin D analogues (Supplementary Fig. 3) (24).

Discussion

In this comparative matched-cohort study, we demonstrate that Japanese patients on HD have overall lower bone turnover based on circulating biomarkers than their European counterparts. Contrary to our hypothesis, we found no indications of a better preserved skeletal PTH responsiveness in Japanese patients, but rather the opposite; Japanese patients had lower levels of bone turnover markers for any given level of PTH.

As expected, there were distinct differences in the parameters of mineral metabolism between the 2 cohorts. Japanese patients had lower levels of PTH and higher levels of phosphate, with a higher prevalence of active vitamin D and calcimimetic use. Thus, better control of secondary hyperparathyroidism was likely achieved by more intensive medical therapy. This may have come at the cost of higher phosphate levels, as active vitamin D analogues increase the gastrointestinal absorption of phosphate (25). A history of parathyroidectomy was more common in the Belgian cohort, which could reflect a longer duration and/or more severe degree of hyperparathyroidism (26), resulting in a greater risk of nodular hyperplasia of the parathyroid glands. However, cautious

interpretation is warranted, as patients with PTH levels below the normal reference range were excluded from analyses, many of whom had undergone a parathyroidectomy. Prevalent cardiovascular disease and previous fractures were more common in Belgian patients, despite matching by major demographic risk factors such as age, sex, diabetes, and dialysis duration.

The majority of Japanese patients (86%-88%) had biochemical bone turnover markers within the normal reference range, whereas this was true for only about two-thirds of the Belgian cohort (57%-67%). Based on previously proposed diagnostic cutoffs of circulating biomarkers (23), high turnover was almost 6 times more common in the Belgian compared to the Japanese cohort (29% vs 5%). On the other hand, half of the Japanese patients were categorized as having low bone turnover, compared to 17% of Belgian patients. The clinical significance of low bone turnover in CKD is debated (27, 28). While both high and low PTH levels have been associated with risk of fracture (6, 7, 29) and cardiovascular events (4, 5), these risks increase linearly for circulating levels of bone turnover markers (30, 31), indicating that the association between low PTH and poor outcomes may be confounded by other factors such as poor nutritional status, chronic inflammation, or the overall burden of comorbidity (27). Of note, fracture incidence among Japanese patients is among the lowest reported when comparing HD cohorts from different regions of the world (32). Demographic differences aside, one might speculate that the stricter PTH control advocated by the Japanese guidelines confers better protection against PTH-driven bone demineralization and consequent fractures.

Our primary hypothesis was that end-organ responsiveness to PTH would be better preserved in Japanese patients, which could provide an argument in favor of lower PTH target levels in this population. However, our results indicate that skeletal responsiveness to PTH is rather *lower* in Japanese compared to European patients on HD, based on lower bone turnover marker levels for any given value of PTH. This PTH hyporesponsiveness became apparent when plotting the relationship between bone turnover markers and PTH, assessing bone turnover markers within different PTH strata, and further matching by PTH levels.

The biological effects of PTH depend on the pattern of release from the parathyroid glands, circulating levels, and end-organ responsiveness (24). The gold standard for evaluating the skeletal response to PTH is by increase in serum calcium in response to PTH infusion, which has been shown to be diminished in CKD (25, 26). We used the bone turnover marker over PTH ratios as a surrogate of skeletal PTH response, relying on the specificity of these biomarkers to reflect the current state of overall skeletal remodeling (17). BALP is an enzyme attached to the cell membrane of osteoblasts that hydrolyzes pyrophosphate into inorganic phosphate (33). It is considered a marker of bone turnover, or more specifically, bone mineralization. TRAP5b is a lysosomal enzyme released from osteoclasts during bone resorption (34). TRAP5b levels are believed to reflect the number of active osteoclasts (35, 36), and systemic levels decrease promptly after initiation of antiresorptive therapy (37). Limitations of BALP include the potential release from sites of nonskeletal calcification (38) and analytical cross-reactivity (10%-20%) with other tissue-specific isoforms of total ALP (39), which could constitute an issue in liver disease or states of marked inflammation (40). Generally, though, BALP and TRAP5b are both accepted as specific biomarkers of skeletal remodeling (41). Neither accumulates in CKD (42), nor do they show substantial variation with dietary intake (43) or time of day (44).

The bone turnover marker over PTH ratios were highest at low-normal levels of PTH and decreased as PTH levels increased. Previous studies similarly indicated a desensitization of the skeleton with severe hyperparathyroidism (45, 46). Wesseling-Perry et al (46) found that the calcemic response to PTH infusion in patients receiving HD was blunted compared to healthy controls when bone turnover was low or normal—but substantially impaired with high turnover bone disease. Downregulation of the PTH1 receptor may be one underlying mechanism, as reduced PTH1R messenger RNA expression was demonstrated in osteoblasts from bone biopsies with high, as compared to normal, bone turnover (47). Such a desensitization could be considered an adaptive response to protect the skeleton from demineralization in severe hyperparathyroidism.

The main determinants of the bone turnover markers over PTH ratios were male sex, obesity, and hyperphosphatemia. A competitive effect of sex hormones on skeletal remodeling could be considered as an explanatory mechanism behind sex-based differences in skeletal PTH responsiveness. Obesity may predispose to metabolic complications such as glucose intolerance and diabetes, which has been linked to a low bone turnover phenotype (48, 49), though to our knowledge, whether bone turnover is lower for a given level of PTH in patients with CKD and diabetes has not been explored. The association with hyperphosphatemia could reflect the severity of hyperparathyroidism—or the intensity of active vitamin D therapy, which itself can modify bone turnover (50). Similar to our findings, a recent cross-sectional study reported that hypocalcemia and hyperphosphatemia were associated with a lower TRAP5b/PTH ratio in Japanese patients, as was the use of calcimimetics and active vitamin D (16). Conclusively, because nationality was not a statistically significant determinant of the bone turnover marker/PTH ratios in adjusted models, we speculate that demography and differences in the use of PTH-lowering therapy (vitamin D receptor activators, calcimimetics) or the results thereof (hyperphosphatemia) may explain the lower skeletal PTH responsiveness in Japanese patients.

We consider the direct comparison in a matched cohort design and the use of bone turnover markers known to be unaffected by kidney function as strengths of this study. Importantly, key parameters of interest—PTH, BALP, and TRAP5b—were measured at a central laboratory, avoiding between-assay variations. Main limitations include the cross-sectional design and a relatively low sample size for a study using biochemical markers as outcome measures. Despite attempts to match the 2 cohorts for major demographic factors, inherent differences between the 2 populations cannot be excluded as potential confounders. The difference in blood sampling is an important consideration, as the timing was standardized with regard to the last dialysis session in the Japanese, while random in the Belgian cohort. The higher phosphate levels in the Japanese cohort should, therefore, be interpreted with caution, as phosphate levels vary between HD sessions (51, 52) and also show a circadian rhythm (45). On the other hand, higher levels of phosphate in Japanese vs European patients on HD have also been shown by others, in large, register-based studies (10). As BALP and TRAP5b show limited circadian (44) and predialysis and postdialysis (53) variation, our main results should not be affected by the difference in sampling time. Finally, we used reference ranges for BALP and TRAP5b as given by the manufacturer, and these were obtained from a Western European population. Conceivably, normal values of bone biomarkers could differ between populations. However, to the best of our knowledge, there are no published normative data for bone turnover markers in Japanese using the IDS-iSYS automated immunoassay.

In conclusion, we found no evidence of better preserved skeletal responsiveness to PTH in Japanese as compared to European patients. The rationale for the current, marked regional differences in PTH treatment targets remains obscure and deserves further attention. It is worth noting that the Japanese treatment targets resulted in normalization of bone turnover markers for a high proportion of patients. Efforts should be made to establish optimal PTH treatment targets with regard to skeletal and cardiovascular health in CKD.

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Disclosures

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Data Availability

Restrictions apply to the availability of the data analyzed in this study to preserve patient confidentiality. Data will be shared on request to the corresponding author with permission from the principal investigators of The Tokai Dialysis Prospective Cohort Study.

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