Journal of Bone and Mineral Metabolism (2019) 37:319–326
https://doi.org/10.1007/s00774-018-0920-5

ORIGINAL ARTICLE

Serum homocysteine levels are affected by renal function during a 3-year period of minodronate therapy in female osteoporotic patients

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Received: 15 October 2017 / Accepted: 4 March 2018 / Published online: 30 March 2018

Abstract
Serum homocysteine is a possible marker to indicate bone quality. However, it is not clear whether changes are seen in serum homocysteine levels with long-term bisphosphonate therapy. We aimed to investigate the factors affecting serum homocysteine levels during a 3-year period of monthly minodronate therapy in osteoporotic women, and to examine if the serum homocysteine levels could reflect some aspects of bone metabolism. The study included 43 patients (age 72.3 ± 7.0 years) undergoing treatment for osteoporosis for the first time (New group) and 35 patients (age 74.4 ± 8.2 years) who switched from alendronate or risedronate to minodronate (Switch group). Minodronate (50 mg/every 4 weeks) was administered for 36 months. Lumbar, femoral neck, and total hip bone mineral densities (BMD), and serum homocysteine levels were monitored at baseline and after 9, 18, 27, and 36 months of treatment. Lumbar BMD increased significantly in both groups (New group 11.4%, Switch group 6.2%). However, femoral neck and total hip BMDs increased only in the New group (femoral neck 3.6%, total hip 4.1%). Serum homocysteine levels increased significantly at 18 and 27 months in all subjects. Multiple linear regression analysis revealed that changes in homocysteine levels during 18, 27, and 36 months significantly correlated with changes in creatinine clearance during the same corresponding periods (18 months: $B = -0.472$, $p = 0.003$; 27 months: $B = -0.375$, $p = 0.021$; 36 months: $B = -0.445$, $p = 0.012$). Thus, serum homocysteine levels possibly reflect renal function instead of bone metabolism during minodronate therapy.

Keywords Bisphosphonate · Bone mineral density · Homocysteine · Minodronate · Osteoporosis

Introduction

Bisphosphonate therapy in patients with osteoporosis results in increased bone mineral density (BMD) through the suppression of bone remodeling, followed by increased mineralization. However, the long-term use of bisphosphonate might compromise bone quality leading to micro-damage of the cortical bone or deterioration of bone micro-architecture, as suggested by iliac crest bone biopsy in clinical studies and histomorphometric analysis in animal models [1–3]. The development of new bone markers for non-invasive detection of bone quality is required. Serum levels of pentosidine are considered a possible candidate for bone quality marker [4]. Previously, we reported changes in BMD and serum levels of pentosidine measured by enzyme-linked immunosorbent assay (ELISA) during a 27-month period of minodronate therapy in female osteoporotic patients [5]. Monthly minodronate treatment increased BMDs of lumbar spine, femoral neck, and total hip in newly treated patients during the 27-month period. Serum levels of pentosidine increased in a time-dependent manner during the study period. However, those levels correlated with estimated glomerular filtration ratio (eGFR), but not with bone parameters. Furthermore, recent study showed that serum levels of pentosidine measured by ELISA were affected by the heating process during measurement procedure [6]. Therefore, we concluded that serum levels of pentosidine measured by ELISA might not reflect bone quality. Homocysteine (Hcy) is an amino acid and a metabolic by-product formed by the conversion of methionine to cysteine. The serum homocysteine level could

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be an independent risk factor for atherosclerotic vascular disease [7]. With respect to bone, Hcy might worsen bone quality by destroying collagen cross-links [8] and causing oxidative damage to the trabecular structure [9]. Serum Hcy levels are associated with the ultrasound parameters of the calcaneus, which may reflect bone quality [10]. Several papers have demonstrated that baseline levels could predict hip or vertebral fractures regardless of BMD, indicating that bone quality could be associated with the occurrence of fragility fractures [11–13]. These earlier reports suggest that the serum levels of Hcy might reflect bone quality during long-term bisphosphonate therapy in osteoporotic patients.

Minodronate (MIN), developed in Japan, is a third-generation amine-group-containing bisphosphonate that contains two imidazole rings, resembling the molecular structure of zoledronate [14]. The farnesyl pyrophosphate synthase activity of MIN is the strongest among the currently available oral bisphosphonates [15]. A daily regimen of MIN has been available in the clinical setting since 2009, although the monthly regimen became available only in 2011. The 3-year results of BMD changes during the daily regimen of MIN were reported by Hagino et al. [16]. However, to our knowledge, the BMD changes induced by the monthly regimen over the course of ≥ 3 years have not yet been reported. It might be valuable to examine how serum Hcy levels change during long-term MIN therapy in osteoporotic patients. To date, longitudinal changes in serum Hcy levels during long-term bisphosphonate therapy have not been reported.

The purpose of the present study was to examine if serum levels of Hcy could reflect some aspect of bone metabolism, by investigating the factors affecting changes in serum Hcy levels during a 3-year period of monthly MIN therapy.

Materials and methods

Study design

This retrospective study was based on a 9-month extension of an original 27-month study of monthly minodronate treatment that was previously reported by authors [5]. In that study, the primary aim was to elucidate the effects of monthly MIN on BMDs and serum pentosidine during the 27 months, and the secondary aim was to identify the factors that predict BMD changes at 27 months in patients who switched to minodronate from alendronate or risedronate. In the current study, we examined changes in serum Hcy levels and factors that affected Hcy levels during 36 months of monthly minodronate therapy.

Patients

Total 78 patients were recruited, those who completed the 36-month monthly minodronate therapy, from the 99 patients from the previous 27-month study of minodronate therapy [5]. The participants were divided into two groups: 43 patients who had never been treated for osteoporosis earlier (New group) and 35 patients who had switched to monthly MIN therapy from alendronate or risedronate (Switch group). The diagnosis of osteoporosis was made according to the criteria proposed by the Japanese Society for Bone and Mineral Research [17]. Drugs other than MIN that may affect bone metabolism were not administered during the study period, with the exception of eldecalcitol. Eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropyloxy) vitamin D_3] (0.75 μg/day) was allowed to be prescribed at the discretion of each physician at 9, 18, or 27 months after the start of the study. Calcium supplementation was not performed throughout the study period. All patients were asked for vitamin B_6, B_{12}, or folate prescription, since those medicines or supplements could affect the serum level of Hcy. The exclusion criteria were as follows: (1) endocrine disorders such as primary hyperparathyroidism; Cushing's syndrome or hypothalamic, pituitary, or gonadal insufficiency; (2) poorly controlled diabetes mellitus (hemoglobin A1c level > 7.5%); (3) alcohol abuse; (4) osteomalacia after gastrectomy; (5) metastatic bone tumors, and (6) renal failure (creatinine clearance < 30 mL/min). Patients were instructed to take one MIN tablet (50 mg) (Astellas Pharma Inc., Tokyo, Japan) orally on an empty stomach before breakfast once every 4 weeks for 36 months. Patients were advised to take eldecalcitol (0.75 μg/day) (Chugai Pharma Co., Ltd., Tokyo, Japan) after breakfast every day. Among the patients who wished to switch to monthly MIN from their previous therapy, no wash-out period was introduced for the previous bisphosphonate.

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the institutional review board at Enshu Hospital (Hamamatsu, Shizuoka, Japan). Written informed consent was obtained from each participant.

Data collection

Blood and urine samples, taken between 9:00 a.m. and 11:00 a.m., were collected at the outpatient ward without overnight fasting. At baseline and at 9, 18, 27, and 36 months after the start of treatment, BMDs of the lumbar spine (L2–4), femoral neck, and total hip; the serum level of intact procollagen type-I N-terminal propeptide

(P1NP); the urinary level of type-I collagen cross-linked N-telopeptide (NTX); and the serum level of Hcy were measured. The BMD was measured using dual-energy X-ray absorptiometry with a QDR Discovery scanner (Hologic Inc., Madison, Bedford, MA, USA). BMD (g/cm^2) was determined for the lumbar spine (L2–4 in anteroposterior projection), femoral neck, and total hip on the left side. If a prosthesis or fracture device was inserted on the left side, the right hip was utilized as the BMD measurement site. The long-term coefficient of variation (CV) determined with the Hologic Phantom was 0.4%. The short-term CV values for lumbar, femoral neck, and total hip BMDs were 1.2, 2.0, and 0.7%, respectively. The serum levels of P1NP were measured using ELISA with an intra-assay CV of 6.6–7.7% and an inter-assay CV < 10% (Orion Diagnostica, Espoo, Finland). The urinary levels of NTX were analyzed using ELISA with an intra-assay CV of 7% and an inter-assay CV of 7–10% (Osteomark: Osteox International, Seattle, WA, USA). The NTX concentration was normalized to that of urinary creatinine. The serum level of total Hcy was measured by high-performance liquid chromatography with an intra-assay CV of 3.0% and an inter-assay CV of 2.0% [18]. All immunoassays were performed by SRL Inc. (Tokyo, Japan). Routine laboratory examinations were also performed, including kidney and liver function tests, as well as the measurement of total protein, albumin, calcium, and phosphate levels. Creatinine clearance was estimated by the Cockcroft and Gault methods [19]. We also identified the patients with a history of fragility factures, including hip, forearm, proximal humerus, pelvis, distal tibia, and symptomatic and non-symptomatic vertebral fractures diagnosed by lateral radiography at the start of the study.

Statistical analysis

Data were analyzed using StatView 5.0 (SAS Institute, Cary, NC, USA). A non-parametric test followed by a Mann–Whitney *U* test was used to compare the numerical data between the two groups. The Chi-square test was used to compare the categorical variables between the groups. Comparisons of related values within groups were conducted using analysis of variance followed by a post hoc Scheffe's test. All values were expressed as mean ± standard deviation, if not otherwise indicated. Lumbar BMD data were excluded in further analyses, if new vertebral fractures between L2 and L4 were observed during the study. To investigate the factors that affected the changes in serum Hcy levels during 18, 27, and 36 months, multiple linear regression analysis was performed. In all analyses, p values < 0.05 were defined as statistically significant.

Results

No significant differences were observed between the groups in terms of age, body mass index, *T* scores of femoral neck and total hip BMDs, serum levels of Hcy, and creatinine

Table 1 Baseline data

Variable	New group	Switch group	*p* value
No.	43	35	
Age (years)	72.3 (7.0)	74.4 (8.2)	0.073
BMI (kg/m^2)	20.8 (3.4)	20.6 (2.8)	0.802
Lumbar BMD *T* score (SD)	− 3.0 (1.2)	− 2.1 (1.5)	0.008
Femoral neck BMD *T* score (SD)	− 2.5 (0.7)	− 2.6 (0.8)	0.778
Total hip BMD *T* score (SD)	− 2.2 (0.9)	− 2.0 (0.9)	0.590
S-P1NP (μg/L)	68.5 (35.9)	29.6 (20.1)	< 0.0001
U-NTX (nmol BCE/mmol Cr)	72.0 (53.5)	24.8 (13.5)	< 0.0001
S-Hcy (μmol/L)	7.9 (1.5)	8.3 (2.5)	0.793
Creatinine clearance (mL/min)	62.0 (15.5)	55.7 (12.6)	0.063
No. of patients with fragility fracture history	17	12	0.633
No. of patients with ELD in addition	21	15	0.598
No. of patients with prior therapy	none	ALN 23 RIS 12	
No. of patients taking vitamin B_6, vitamin B_{12}, or folate supplements	3	2	0.820

The numbers in parentheses indicate the standard deviation

BMI body mass index, *BMD* bone mineral density, *S-P1NP* serum intact procollagen type-I N-terminal propeptide, *U-NTX* urinary N-terminal telopeptide of type-I collagen, *BCE* bone collagen equivalent, *Cr* creatinine, *S-Hcy* serum total homocysteine, *No.* number, *ELD* eldecalcitol, *ALN* alendronate, *RIS* risedronate

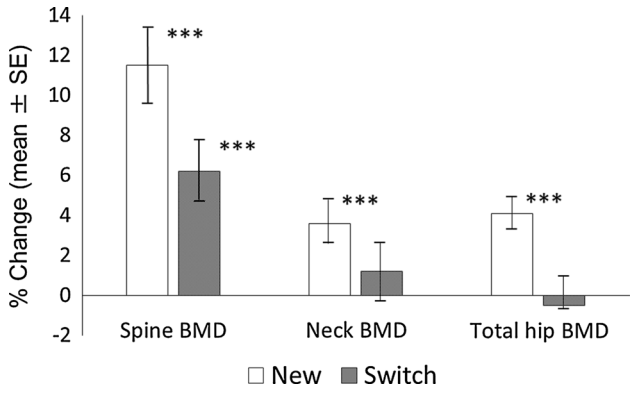

Fig. 1 Percentage changes (mean ± SE) in lumbar, femoral neck, and total hip bone mineral density (BMD) during 36 months in the New and the Switch groups. ***$p < 0.001$ compared to baseline values

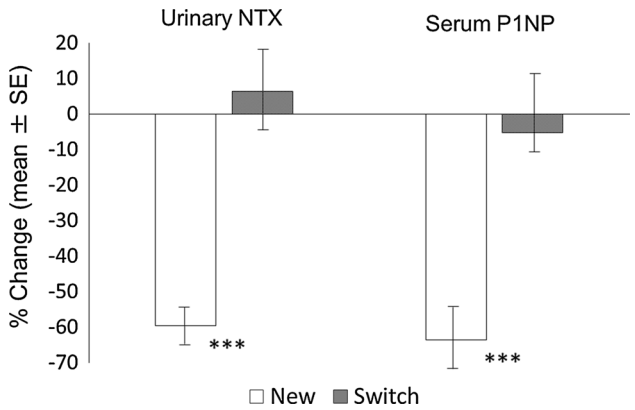

Fig. 2 Percentage changes (mean ± SE) in urinary type-I collagen cross-linked N-telopeptide (NTX) and serum intact procollagen type-I N-terminal propeptide (P1NP) during 36 months in the New and the Switch groups. ***$p < 0.001$ compared to baseline values

clearance at baseline (Table 1). Reflecting the results of prior therapy for osteoporosis, the lumbar *T* score was higher and the urinary NTX and serum PINP levels were lower in the Switch group than those in the New group. The duration of the previous treatment in the Switch group was 40.4 ± 16.4 months for alendronate and 37.8 ± 19.9 months for risedronate. There were no significant differences in the number of the patients taking vitamin B_6, B_{12}, or folate between the groups. During the 3-year study period, lumbar BMD increased significantly compared to the baseline values in both groups (New group 11.4%, Switch group 6.2%). However, femoral neck and total hip BMDs increased only in the New group (femoral neck 3.6%, total hip 4.1%) (Fig. 1). Urinary levels of NTX and serum levels of P1NP decreased significantly in the New group (urinary NTX − 59.6%, serum P1NP − 63.5%) (Fig. 2). In the Switch group, neither the urinary levels of NTX nor the serum levels of P1NP changed significantly during the study period. Creatinine clearance decreased at 27 and 36 months compared to the baseline values in both groups (Fig. 3). Mean total serum Hcy levels were within the normal range (3.7–13.5 μmol/L) throughout the study period (Fig. 4a, upper panel). No significant change in the Hcy level was observed during the 36 months in either the New or the Switch group (Fig. 4a, b, upper panels). However, in all subjects, the levels of Hcy at 18 and 27 months were significantly higher than the baseline values (Fig. 4a, b, lower panels). Multiple linear regression analyses among all subjects revealed that the changes in total Hcy levels during the 18, 27, and 36 months of MIN therapy were affected by changes in creatinine clearance during the same corresponding periods (Table 2). Neither changes in the BMDs nor bone turnover markers affected the changes in serum Hcy levels.

Discussion

This study is based on the data from a 9-month extension of the original 27-month minodronate therapy [5]. First, we compared the changes in BMDs and bone turnover markers during 36 months of minodronate therapy with those reported in the original 27-month study. Then, we discussed the changes in the serum Hcy levels.

More increase in lumbar spine, femoral neck, and total hip BMDs during the 36-month period was observed compared

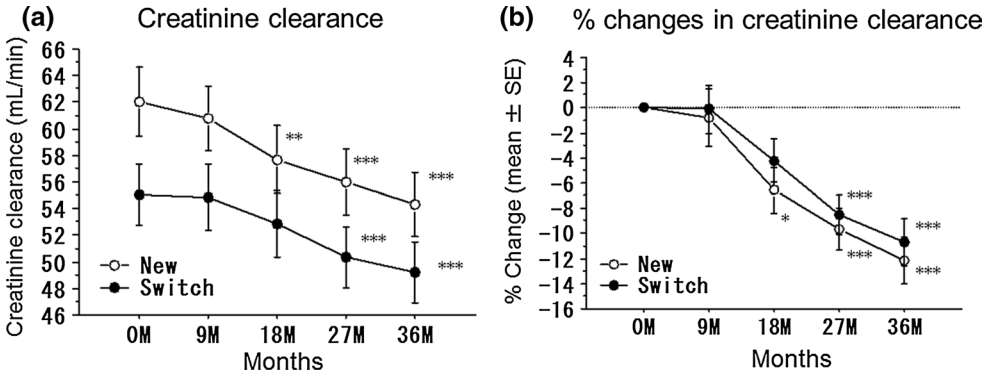

Fig. 3 **a** Longitudinal changes (mean ± SE) and **b** percentage changes (mean ± SE) in creatinine clearance in the New and the Switch groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ compared to baseline values

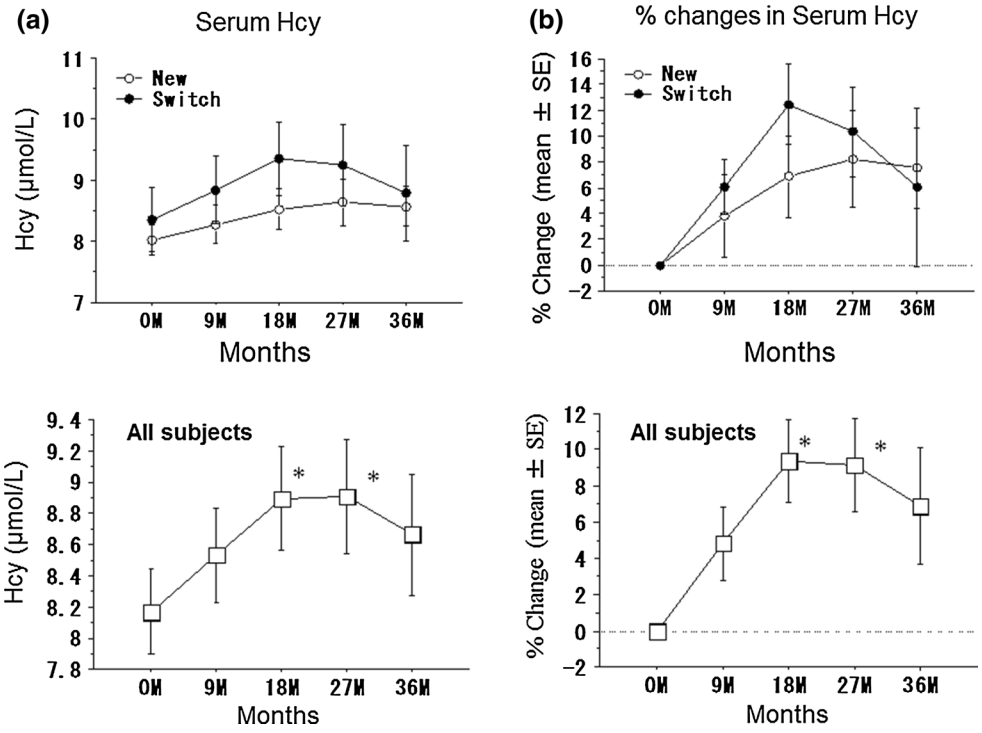

Fig. 4 **a** Longitudinal changes (mean ± SE) and **b** percent changes (mean ± SE) in serum total homocysteine (Hcy) level in the New and the Switch groups (upper panel) and in all subjects (lower panel). *$p < 0.05$ compared to baseline values

Table 2 Multiple linear regression of percent changes in serum Hcy level during 18, 27, and 36 months (M) and changes in bone parameters in the same periods

	Hcy 18-month change			Hcy 27-month change			Hcy 36-month change		
	B	*p* value	95% CI	*B*	*p* value	95% CI	*B*	*p* value	95% CI
Age (years)	−0.157	0.276	−1.121 to 0.329	−0.055	0.733	−0.980 to 0.695	−0.097	0.523	−1.134 to 0.585
BMI (kg/m^2)	−0.008	0.955	−1.554 to 1.470	0.034	0.823	−1.507 to 1.884	0.012	0.935	−1.672 to 1.814
Lumbar BMD change (%)	−0.306	0.053	−1.002 to 0.007	0.013	0.937	−0.522 to 0.564	0.030	0.832	−0.379 to 0.469
Neck BMD change (%)	−0.116	0.445	−1.459 to 0.652	−0.329	0.068	−2.070 to 0.076	−0.035	0.841	−1.234 to 1.009
Total hip BMD change (%)	−0.056	0.695	−1.188 to 0.800	0.238	0.175	−0.495 to 2.450	0.125	0.450	−0.840 to 1.859
Urinary NTX change (%)	0.116	0.572	−0.102 to 0.183	0.267	0.201	−0.045 to 0.207	−0.185	0.384	−0.213 to 0.084
Serum P1NP change (%)	−0.046	0.819	−0.218 to 0.173	−0.159	0.459	−0.276 to 0.127	0.343	0.095	−0.020 to 0.250
Ccr change (%)	−0.472	0.003	−1.207 to −0.271	−0.375	0.021	−1.348 to −0.114	−0.445	0.012	−1.381 to −0.183

Hcy homocysteine, *B* standardized coefficients, *CI* confidence interval, *BMI* body mass index, *BMD* bone mineral density, *NTX* N-terminal telopeptide of type-I collagen, *P1NP* intact procollagen type-I N-terminal propeptide, *Ccr* creatinine clearance

to their increase during the 27-month period in the New group (36 months: lumbar spine, 11.4%; femoral neck, 3.6%; total hip, 4.1% vs. 27 months: lumbar spine, 9.1%; femoral neck, 3.2%; total hip, 3.1%). According to Hagino et al, lumbar BMD increased up to 11% during a 3-year period of daily MIN therapy in post-menopausal osteoporotic women [16]. The results of this study, in which a monthly regimen of MIN treatment was used, are in line with these previous results. On the other hand, suppression of bone markers during the 36-month period was almost the same as observed those during the 27-month therapy (36 months: urinary NTX, − 59.6%; serum P1NP, − 63.5% vs. 27 months: urinary NTX, − 57.0%; serum P1NP, − 64.5%). Excessive suppression of bone turnover might not occur even in long-term minodronate therapy. In the Switch group, profiles of changes in BMDs and bone turnover markers were the same as those seen in the original 27-month study. Only lumbar BMD increased during the 36 months when alendronate or risedronate was replaced by monthly minodronate.

The novel finding of the present study is that the serum levels of Hcy increased significantly at 18 and 27 months during MIN administration compared to the baseline values, and the increase of Hcy is possibly attributed not to the deterioration of bone quality but to the decreased renal function.

Several histomorphometric studies of animal models have indicated that bone micro-damage is induced by high doses of MIN, but optimal doses appeared to maintain or even ameliorate bone micro-architecture and strength [20–22]. On the contrary, recent reports demonstrated that even optimal doses of alendronate after long-term administration result in an accumulation of micro-cracks and perforations in human cancellous bone [23, 24]. There were no significant differences in baseline levels of serum Hcy between the two groups in our study. If the serum levels of Hcy were associated with bone quality, the baseline levels in the Switch group would be higher than those in the New group, since alendronate or risedronate was already administered for approximately 39.5 months. In the present study, multiple lineal regression analyses showed that changes in Hcy levels at 18, 27, and 36 months of MIN administration correlated well with changes in renal function during the same corresponding period, but not with anthropometric parameters or changes in bone markers including the BMDs. To the best of our knowledge, only one previous paper has demonstrated longitudinal changes in serum Hcy levels during bisphosphonate therapy in osteoporotic patients. Tariq et al. reported no significant changes in serum Hcy levels during a 6-month period of ibandronate therapy in 41 post-menopausal women with osteoporosis [25]. Concerning the nature of the accumulation of bisphosphonate in bone, longer periods of study may be necessary to verify if the serum levels of Hcy can reflect bone quality. Considering minor or no adverse effects of MIN on bone quality as evaluated by the previous histomorphometric studies and the strong association of serum Hcy level with renal function as per our results, we conclude that the serum level of Hcy possibly does not reflect bone quality but rather reflects renal function. Histomorphometric analyses of bone biopsies during long-term MIN therapy will be necessary to confirm these conclusions.

Till date, it has been reported that the serum level of Hcy significantly correlates with renal failure and increases in the early phase of diminished renal function [26, 27]. According to Gerdhem et al., serum creatinine levels in the highest quintile of Hcy levels were significantly higher than the values in all other participants among 996 healthy women aged ≥ 75 years [28]. Fujita et al. reported that the eGFR was inversely associated with serum Hcy levels in 1,477 healthy Japanese men aged ≥ 65 years [29]. Both studies were cross-sectional in design. However, the serum Hcy levels could be affected by renal function to a greater degree if studied in a longitudinal manner. LeBoff et al. reported that high serum Hcy levels were associated with an increased risk of hip fracture. However, serum levels of cystatin-C, which is involved in renal function, were also elevated in the high-risk group. The authors concluded that the possible involvement of renal function in the relationship between Hcy and hip fracture risk should be taken into consideration [30]. In the current study, creatinine clearance significantly decreased in both the groups during the 3-year treatment period by 11–13% compared to the baseline values. In a review article, Miller described that in the HORIZON-Recurrent Fracture Trial, which included 2000 elderly male and female patients, the eGFR decreased by 7.3%, during the 3-year period, in the placebo group [31]. The decrease in creatinine clearance during the 3-year period in our study was greater than the results using eGFR. Creatinine clearance is a better indicator for renal function, because not only the age but also the body weight is taken into consideration for the estimation of renal function. The decreased creatinine clearance reported in the present study was probably caused by aging rather than the kidney toxicity of MIN. When the serum level of Hcy is measured during bisphosphonate therapy to examine whether they reflect BMD, bone turnover or bone quality, and renal function (including creatinine clearance or eGFR) should be assessed simultaneously.

There are several limitations to the present study. A major limitation is that we included only a small number of participants. Second, the Switch group consisted of the patients who wanted to switch from alendronate or risedronate regardless of BMDs or the level of bone markers. Therefore, the baseline lumbar *T* score of BMD and bone markers were significantly different between the groups. Third, the conclusion that the serum level of Hcy may not reflect bone quality should be interpreted cautiously, because histomorphometric analyses including a bone biopsy were not performed. Bone biopsies will be necessary for reliable conclusion. Fourth, the baseline levels of creatinine clearance in both groups were still low, although the subjects whose creatinine clearance levels were below 30 mL/min were excluded. Therefore, it cannot be confirmed that our results are applicable to younger subjects whose renal functions are better. Fifth, serum Hcy levels are affected by daily folate, and B_6 or B_{12} intake [32]. We asked all subjects for their prescription of these drugs or supplements, and the numbers of the patients taking these supplements were not different between the groups. No evaluation of dietary nutrient intake was performed in this study. However, blood and urine samples were collected between 9:00 a.m. and 11:00 a.m. to minimize the effect of the diet.

In conclusion, the serum level of Hcy may not reflect bone metabolism possibly including bone quality, but may reflect renal function during a 3-year period of monthly MIN therapy.

Acknowledgements The authors would like to thank Yoshiaki Ito, Yasuyuki Takabayashi, and Yasuhide Suzuki, who are laboratory technicians at Enshu Hospital, for their assistance with blood sample preparation.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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