Effects of PTH and Alendronate on Type I Collagen Isomerization in Postmenopausal Women With Osteoporosis: The PaTH Study

Patrick Garnero,^{1,2} Doug C Bauer,³ Emmanuel Mareau,² John P Bilezikian,⁴ Susan L Greenspan,⁵ Clifford Rosen,⁶ and Dennis Black³

ABSTRACT: Fracture efficacy of PTH and alendronate (ALN) is only partly explained by changes in BMD, and bone collagen properties have been suggested to play a role. We analyzed the effects of PTH(1-84) and ALN on urinary α/β CTX ratio, a marker of type I collagen isomerization and maturation in postmenopausal women with osteoporosis. In the first year of the previously published PaTH study, postmenopausal women with osteoporosis were assigned to PTH(1-84) (100 μ g/d; $n = 119$), ALN (10 mg/d; $n = 60$), or PTH and ALN together ($n = 59$). We analyzed patients on ALN alone ($n = 60$) and a similar number of patients assigned to PTH alone ($n = 63$). During the second year, women on PTH in the first year were reallocated to placebo ($n = 31$) or ALN ($n = 32$) and women with ALN continued on ALN. During the first year, there was no significant change in α/β CTX ratio with PTH or ALN. At 24 mo, there was a marked increase of the α/β CTX ratio in women who had received PTH during the first year, followed by a second year of placebo (median: +45.5, $p < 0.001$) or ALN (+55.2%, $p < 0.001$). Conversely, the α/β CTX ratio only slightly increased (+16%, $p < 0.05$) after 2 yr of continued ALN. In conclusion, treatment with PTH(1-84) for 1 yr followed by 1 yr of placebo or ALN may be associated with decreased type I collagen isomerization. The influence of these biochemical changes of type I collagen on bone fracture resistance remains to be studied. *J Bone Miner Res* 2008;23:1442–1448. Published online on April 21, 2008; doi: 10.1359/JBMR.080413

Key words: osteoporosis, type I collagen, PTH, alendronate, biochemical markers

INTRODUCTION

OSTEOPOROSIS IS A common age-related disease characterized by increased skeletal fragility leading to fracture. The bone fracture resistance depends on different parameters including its mass and geometry, its microarchitecture, and the material properties of the bone matrix itself.⁽¹⁾ Bone matrix can be considered a composite material, comprised of water, mineral, and organic phases. The mineral phase largely accounts for the stiffness of bone,^(2,3) whereas the organic phase, mainly constituted of type I collagen, provides bone its ductility and toughness (i.e., its ability to undergo deformation and absorb energy after it begins to yield).^(4,5) Although clinical assessment of BMD by DXA, which largely reflects the mineral phase, is the current gold standard for diagnosis of osteoporosis, ~50% of postmenopausal women with incident fracture have BMD levels above the WHO criteria for osteoporosis,^(6,7) suggesting that factors not reflected in an BMD measurement, such as the organic phase of bone, may contribute to skeletal fragility.

Posthoc analyses of prospective clinical trials have shown

that the observed changes in BMD with anti-catabolic therapies including the bisphosphonates alendronate and risedronate and the selective estrogen receptor modulator raloxifene account for only a small proportion of their anti-fracture efficacy.⁽⁸⁾ Treatment with PTH markedly increases bone tissue mass primarily by increasing bone modeling.^(9,10) The mechanisms involved in the anti-fracture efficacy of teriparatide [PTH(1-34)] and full length PTH(1-84) are still poorly understood, but one study showed that the increase in BMD with teriparatide explains <40% of its efficacy to reduce vertebral fracture risk.⁽¹¹⁾ Thus, changes in BMD with PTH account for only part of their efficacy to reduce fracture risk.

Several animal and cadaveric human studies have shown that modifications of the bone matrix properties including alterations in collagen cross-linking and matrix maturation may contribute to bone fracture resistance including bone strength and toughness.^(12–22) Maturation of collagen molecules in bone matrix includes the formation of enzymatic cross-links within the N- and C-telopeptides—which is initiated by the conversion of lysine and hydroxylysine residues through the activity of the lysyl oxidase—and the non-enzymatic advanced glycation endproducts (AGEs), which occur spontaneously in the presence of extracellular sugars. In contrast to enzymatic telopeptide cross-links whose con-

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¹INSERM Research Unit 664, Lyon, France; ²Synarc, Molecular Markers, Lyon, France; ³UCSF, San Francisco, California, USA; ⁴Columbia University, New York, New York, USA; ⁵University of Pittsburgh, Pittsburgh, Pennsylvania, USA; ⁶St Joseph Hospital, Bangor, Maine, USA.

centration in bone tissue plateaus with skeletal maturity, AGEs including the cross-link pentosidine accumulates with age in human cortical bone.⁽²³⁾ In addition to these two types of collagen cross-linking molecules, type I collagen can also undergo a spontaneous isomerization of the aspartic acid (D) residue within the EKAHDGGR sequence (CTX motif) of the C-telopeptide of α 1 chains.⁽²⁴⁾ In the native newly synthesized collagen, the aspartic acid (D) is linked to the adjacent glycine (G) residue through its carboxyl group in position α . After isomerization, D is linked to adjacent G residue through its carboxyl group in position β .^(24,25) In contrast to the enzymatic telopeptide cross-links and some nonenzymatic AGEs (e.g., pentosidine), this spontaneous nonenzymatic isomerization process does not lead per se to the formation of cross-links between adjacent collagen molecules. However, isomerization does alter the conformation of the type I collagen C-telopeptide by introducing a kink in the peptide backbone.^(24,25) The kinetic of aspartic acid isomerization at 37°C has been studied in vitro using synthetic CTX peptides and immature fetal bovine bone collagen extract, which consists mainly of α CTX isomers.⁽²⁵⁾ At equilibrium of the reaction, ~20% of CTX peptide remains on its original α form and 80% is β isomerized.⁽²⁵⁾ In case of high bone remodeling observed, for example, in fetal tissue⁽²⁵⁾ and in patients with Paget's disease of bone,⁽²⁶⁾ the steady state of the reaction can not be achieved, leading to displacement of the equilibrium in favor of the α CTX form. Thus, in contrast to the nonenzymatic cross-links AGEs, which accumulate with aging in bone tissue, the relative concentration of isomerized β CTX versus α CTX can not exceed the level reached at the equilibrium of the kinetic of isomerization.⁽²⁵⁾ Although the exact mechanisms relating isomerization of type I collagen to bone fracture resistance have not been determined, ex vivo studies of human vertebral bone have shown that the extent of CTX isomerization was associated with alterations of biomechanical properties of bone tissue, in part independently of BMD.^(22,26) In vivo, the relative proportion of β isomerized type I collagen C-telopeptide can be assessed by immunoassays using conformational antibodies recognizing specifically the α or the β isomers of CTX fragments excreted in urine during bone resorption. Because these are sandwich immunoassays, they are recognizing the two CTX peptides originating from one of the two α 1 chains of a type I collagen molecule linked together through their lysine (K) residue and are thus referred to as $\alpha\alpha$ CTX and $\beta\beta$ CTX, respectively. In untreated postmenopausal women, it has been shown that subjects with a urinary $\alpha\alpha/\beta\beta$ CTX ratio in the highest quartile had an increased risk of incident fracture, which remained significant after adjustment for BMD and bone turnover.⁽²⁷⁾ The effects of PTH on urinary $\alpha\alpha/\beta\beta$ CTX ratio are currently unknown.

The aim of this study was to investigate the effects of full-length PTH and alendronate type I collagen isomerization as reflected by changes in the urinary $\alpha\alpha/\beta\beta$ CTX ratio in postmenopausal with osteoporosis. We hypothesized that this ratio might change with PTH adminis-

tration and be different from alendronate in view of the actions of PTH to increase and alendronate to decrease bone turnover.

MATERIALS AND METHODS

We studied women from the previously described PaTH study.^(10,28) Briefly, 238 postmenopausal women 55–89 yr of age with osteoporosis were included. Osteoporosis was defined by a BMD T-score at the femoral neck, total hip, or spine below –2.5 or a BMD T-score below –2.0 and at least one clinical risk factor (age $\geq$ 65 yr, history of postmenopausal fracture, maternal history of hip fracture). During the first year of PaTH, women were randomly assigned to take PTH(1-84) alone (100 μ g/d, SC injection; NPS Pharmaceuticals; n = 119), alendronate alone (10 mg/d PO, Fosamax; Merck; n = 60), or the combination of alendronate and PTH (n = 59). During the second year of PaTH,⁽²⁸⁾ women on PTH alone in the first year were randomly reallocated to placebo or alendronate, whereas the two other groups received alendronate alone. All women also received 500 mg of calcium carbonate and 400 UI of vitamin D.

In this study, we analyzed 63 women randomly selected from 119 women in the PTH-alone group and 60 women in the alendronate-alone group and examined markers over 2 yr. Baseline demographics and spine and hip BMD values of the PTH subset analyzed in this study were similar and not significantly different from that of the whole PTH group (data not shown).

Measurements of urinary $\alpha\alpha$ CTX and $\beta\beta$ CTX

Fasting second morning void urine samples were collected in all women at baseline and 1, 3, 12, and 24 mo after initiating therapy. All urine samples were stored frozen at a temperature below –70°C until assay.

Urinary $\alpha\alpha$ CTX was measured by a two-site ELISA using two monoclonal antibodies raised against the native EKAHDGGR sequence (Alpha Crosslaps; Nordic Biosciences, Herlev, Denmark). In our laboratory, intra-assay CVs evaluated by measuring three different urine samples 20 times in the same run ranged from 2% to 2.9%. Inter-assay precision variation evaluated by measuring three different urine samples in 10 different runs ranged from 3.7% to 11.5%. Urinary $\beta\beta$ CTX was measured using a two-site ELISA using two monoclonal antibodies raised against the β -isomerized EKAH β DGGR sequence (Serum Crosslaps; Nordic Biosciences). Because this assay was initially developed for serum measurements, we evaluated its analytical accuracy by successive dilution of three urine samples up to 1/32. The dilution recovery ranged from 98% to 115%, showing linearity of measurements for urine determination. The intra-assay variation evaluated by measuring three different urine samples 20 times in the same run ranged from 0.7% to 1.0%. Interassay precision variation evaluated by measuring three different urine samples in 10 different runs ranged from 3.8% to 7.0%.

The cross-reactivity of these two assays for their respective counterpart sequences was <2%.

Urinary $\alpha\alpha$ CTX and $\beta\beta$ CTX levels were corrected by

TABLE 1. BASELINE CHARACTERISTICS OF POSTMENOPAUSAL WOMEN IN THE PTH(1-84) AND ALENDRONATE GROUP

Variable	PTH(1-84) (n = 63)	Alendronate (n = 60)	p
Age (yr)	68.7 ± 7.7	70.4 ± 7.0	0.34
Age at menopause (yr)	47.1 ± 6.6	48.3 ± 5.2	0.53
Body mass index (kg/m ²)	25.81 ± 4.5	25.2 ± 4.53	0.49
Lumbar spine BMD (g/cm ²)	0.76 ± 0.10	0.78 ± 0.13	0.99
Total hip BMD (g/cm ²)	0.70 ± 0.09	0.71 ± 0.09	0.82

urinary creatinine (Cr) measured by a standard colorimetric assay, and results are expressed in $\mu\text{g}/\text{mmol Cr}$.

Urinary $\alpha\alpha/\beta\beta$ CTX ratio in young adults was determined from 30 healthy premenopausal women 35–45 yr of age. All women were menstruating regularly, and none had disease or treatment that could interfere with bone metabolism.

Markers of bone turnover

Serum type I collagen N-terminal propeptide (PINP), a marker of bone formation, and serum $\beta\beta$ CTX, a marker of bone resorption, were measured using the automated analyzer (total PINP and serum Cross-laps, respectively, ELECSYS; Roche Diagnostics) as previously reported.^(10,28)

BMD

Areal BMD at the lumbar spine and total hip was assessed by DXA (Hologic QDR 4500A or Delphi densitometers) at baseline and 12 and 24 mo as previously described.^(10,28)

Statistical analyses

All data are shown as mean (SD) unless otherwise specified. Because changes of biochemical markers were not normally distributed and normal distribution could not be obtained by logarithmic transformation, median and distribution-free 95% CIs are reported for changes in the levels of bone markers as previously used in PaTH.⁽¹⁰⁾ Student's *t*-test was used to compare baseline demographic variables and BMD between the PTH and alendronate groups. Nonparametric Wilcoxon tests were used to compare the changes of $\alpha\alpha$ CTX/Cr, $\beta\beta$ CTX/Cr, and the ratio $\alpha\alpha/\beta\beta$ CTX between the alendronate and PTH groups as previously performed to analyze changes in conventional markers of bone turnover in PaTH.⁽¹⁰⁾ Correlations between urinary $\alpha\alpha$ CTX/Cr, $\beta\beta$ CTX/Cr, and the ratio $\alpha\alpha/\beta\beta$ CTX and BMD or markers of bone turnover were assessed by nonparametric Spearman Rank correlation analyses.

RESULTS

Baseline characteristics of the patients in the PTH- and alendronate-treated group

At baseline, there was no significant difference between the PTH- and alendronate-treated women for any demographic variables or spine and hip BMD (Table 1). There was also no significant difference in serum PINP, serum $\beta\beta$ CTX, urinary $\alpha\alpha$ CTX/Cr, and the $\alpha\alpha/\beta\beta$ CTX ratio, whereas urinary $\beta\beta$ CTX/Cr values were slightly higher in the PTH group (Table 2).

TABLE 2. BASELINE LEVELS OF BIOCHEMICAL MARKERS OF BONE TURNOVER IN THE PTH(1-84) AND ALENDRONATE GROUP

Marker	PTH(1-84) (n = 63)	Alendronate (n = 60)	p
Serum PINP (ng/ml)	55.9 ± 28.8	50.9 ± 25.3	0.31
Serum $\beta\beta$ CTX (ng/ml)	0.39 ± 0.20	0.36 ± 0.18	0.52
Urinary $\alpha\alpha$ CTX/Cr ($\mu\text{g}/\text{mmol Cr}$)	0.91 ± 0.56	0.79 ± 0.37	0.47
Urinary $\beta\beta$ CTX/Cr ($\mu\text{g}/\text{mmol Cr}$)	2.72 ± 1.41	2.17 ± 1.27	0.03
$\alpha\alpha/\beta\beta$ CTX ratio	0.36 ± 0.19	0.41 ± 0.20	0.13

At baseline, in all subjects, urinary $\alpha\alpha$ CTX/Cr was significantly correlated with urinary $\beta\beta$ CTX/Cr ($r = 0.79$, $p < 0.0001$). The urinary $\alpha\alpha/\beta\beta$ CTX ratio did not correlate significantly with age ($p = 0.94$), body mass index ($p = 0.09$), serum PINP ($p = 0.75$), serum $\beta\beta$ CTX ($p = 0.75$), or spine BMD ($p = 0.91$). There was a weak negative correlation between urinary $\alpha\alpha/\beta\beta$ CTX ratio and total hip BMD ($r = -0.20$, $p = 0.03$).

One-year changes of urinary $\alpha\alpha$ CTX/Cr, $\beta\beta$ CTX/Cr, and $\alpha\alpha/\beta\beta$ CTX ratio with PTH and alendronate

Alendronate induced a rapid decrease in both urinary $\alpha\alpha$ CTX/Cr and $\beta\beta$ CTX/Cr in 1 mo (median: -58% and -60% , $p < 0.001$ versus baseline for $\alpha\alpha$ CTX/Cr and $\beta\beta$ CTX/Cr, respectively), which was maintained during the 1 yr of treatment. Conversely, PTH induced a significant increase of $\alpha\alpha$ CTX/Cr and $\beta\beta$ CTX/Cr, which was significant only after 3 mo (median: $+27\%$ and $+43\%$ for $\alpha\alpha$ CTX/Cr and $\beta\beta$ CTX/Cr, respectively; $p < 0.05$), and values further increased at 12 mo (median: $+84$ and $+95\%$, respectively; $p < 0.001$ versus baseline; Fig. 1). At all time points, changes in $\alpha\alpha$ CTX/Cr and $\beta\beta$ CTX/Cr were significantly different between the PTH and alendronate groups ($p < 0.0001$).

There was a slight but nonsignificant ($p > 0.5$) increase in the $\alpha\alpha/\beta\beta$ CTX ratio with either PTH or alendronate at 1 yr. Changes in the $\alpha\alpha/\beta\beta$ CTX ratio did not differ between PTH- and alendronate-treated women at all time points during the first year ($p > 0.30$; Fig. 1).

Changes in urinary $\alpha\alpha/\beta\beta$ CTX ratio at 1, 3, and 12 mo did not significantly correlate with the corresponding changes of serum PINP and serum $\beta\beta$ CTX both in the PTH and alendronate groups. There was also no significant association between changes in $\alpha\alpha/\beta\beta$ CTX ratio at 12 mo and changes in spine or hip BMD at 12 mo with either PTH or alendronate.

Changes in urinary $\alpha\alpha$ CTX/Cr, $\beta\beta$ CTX/Cr, and $\alpha\alpha/\beta\beta$ CTX ratio after 2 yr of continued alendronate, 1 yr of placebo after 1 yr of PTH, and 1 yr of alendronate after 1 yr of PTH

During the second year of PaTH, patients on alendronate during the first year continued with alendronate, whereas patients on PTH in year 1 received either placebo ($n = 31$) or alendronate ($n = 32$).

In the 2-yr continued alendronate group, urinary $\alpha\alpha$ CTX/Cr and $\beta\beta$ CTX/Cr remained stable (Fig. 2). One year

FIG. 1. Changes of urinary α CTX/cr, β CTX/Cr, and ratio α/β CTX in postmenopausal women treated with PTH(1-84) or alendronate for 1 yr. The graphs show the median and the 95% CIs of the percent changes from baseline.

FIG. 2. Changes of urinary α CTX/cr, β CTX/Cr, and ratio α/β CTX in postmenopausal women treated with 2 yr of continued alendronate, 1 yr of PTH, followed by 1 yr of placebo and 1 yr of PTH followed by 1 yr of alendronate. The graphs show the median and 95% CIs of the percent changes from baseline.

of placebo after 1 yr of PTH induced a decrease in α CTX/Cr and β CTX/Cr, which returned to baseline levels after 24 mo (Fig. 2). One year of alendronate after 1 yr of PTH induced a marked decrease in α CTX/Cr and β CTX/Cr, and levels reached after 24 mo were similar to those observed after 2 yr of continued alendronate (Fig. 2).

One year of placebo or alendronate after 1 yr of PTH was associated with a marked increase in the α/β CTX ratio (median increase at 24 mo versus baseline: +45.5, $p < 0.001$ and +55.2, $p < 0.001$ for placebo and alendronate, respectively); however, there was large interpatient variability (Fig. 2). In contrast, after 2 yr of continued alendronate, the α/β CTX ratio increased only slightly (+16%, $p < 0.05$ versus baseline; Fig. 2). The percentage increase of α/β CTX ratio at 2 yr was significantly greater in patients receiving PTH for 1 yr followed by 1 yr of alendronate compared with 2 yr of continued alendronate ($p = 0.04$).

At baseline before treatment, the average absolute level of the α/β CTX ratio in postmenopausal women in the PaTH study was significantly higher than values in premenopausal controls (mean $\pm$ SD: 0.37 ± 0.16 versus 0.23 ± 0.045 , $p < 0.0001$). At 24 mo, the α/β CTX ratio after 1 yr of PTH followed by 1 yr of alendronate was significantly higher than after 2 yr of continued alendronate (0.59 ± 0.24 versus 0.44 ± 0.21 , $p < 0.01$). Subjects receiving PTH for 1 yr

followed by placebo for a second year also had a higher α/β CTX ratio than patients receiving 2 yr of continued alendronate, although the difference did not reach statistical significance ($p = 0.08$). In the combined groups of 1 yr of PTH followed by 1 yr of placebo or alendronate, the α/β CTX at 24 mo was also significantly higher ($p < 0.01$) than in women receiving 2 yr of alendronate.

DISCUSSION

In this study, we report changes in urinary α/β CTX ratio—a possible biochemical marker of bone type I collagen maturation—in a randomized head to head comparison of full-length PTH and alendronate in postmenopausal women with osteoporosis. After 1 yr of either alendronate or PTH alone, there was no significant change in the α/β CTX ratio. However, at 24 mo, patients who had received PTH during the first year and then placebo or alendronate for a second year showed a marked increase in the α/β CTX ratio, suggesting that PTH therapy may result in overall decreased bone collagen maturation.

At baseline, there was no significant association between the urinary α/β CTX ratio and the overall rate of bone turnover or spine BMD. These findings support the view that, in untreated postmenopausal women, the α/β CTX

ratio provides information that is largely independent of bone turnover rate and BMD. These results are also in agreement with the previously reported BMD and bone turnover independent associations of the α/β CTX ratio with fracture risk in untreated postmenopausal women.⁽²⁷⁾

PTH administration induced a marked increase in bone formation and synthesis of new type I collagen molecules as shown by the marked elevation of serum PINP.⁽¹⁰⁾ Newly synthesized collagen molecules are incorporated into bone matrix in a native α CTX conformation. If this new bone collagen matrix is resorbed before the kinetic of isomerization reaches equilibrium, one would expect an increase in the urinary α/β CTX ratio. In our study, we observed no significant changes in the α/β CTX ratio during the first year of PTH treatment. These findings suggest that type I collagen degradation products excreted in the urine during the first year of PTH may arise predominantly from resting bone that was formed before initiating therapy. Alternatively, the isomerization of collagen formed during PTH treatment reached equilibrium before being degraded. In support of the former hypothesis, an in vitro study using human osteoclastic cells cultured on bovine cortical bone slices from animals of different ages suggested that aged bone matrix, characterized by a higher degree of type I collagen isomerization, is preferentially resorbed.⁽²⁹⁾

One year of placebo or alendronate after 1 yr of PTH was, however, associated with a marked increase in the α/β CTX ratio, whereas it remains almost unchanged with continued 2 yr of alendronate. It is possible that during the second year of the study, part of the bone matrix that has been formed during the first year of PTH is becoming degraded. Because bone matrix formed under PTH therapy may contain collagen molecules not fully isomerized, the urinary α/β CTX ratio would increase. Fourier transform infrared imaging (FTIRI) of iliac crest bone sections of patients treated with teriparatide [PTH(1-34)], obtained for the majority after 2 yr of treatment, has shown a lower mature/immature collagen cross-link ratio (pyridinoline/dehydrodihydroxylysinorleucine ratio), suggesting decreased collagen matrix maturation with long-term PTH therapy.⁽³⁰⁾ However, because the FTIRI enzymatic cross-link ratio reflects a collagen maturation process different than aspartic acid isomerization, data obtained by these two parameters cannot be compared directly. Whether continued 2-yr PTH would result in an even higher increase of urinary α/β CTX ratio remains to be studied.

Although the effects of bisphosphonates on mineral maturity/crystallinity have been studied in several studies using various techniques,⁽³¹⁾ much less attention has been directed to their influence on collagen properties. An FTIRI study analyzed the ratio between mature and immature enzymatic collagen cross-links on paired iliac crest biopsies from women with postmenopausal osteoporosis taken after 3 and 5 yr of treatment with placebo or risendronate.⁽³²⁾ At the bone-forming sites, characterized by evidence of primary mineralization, there was no change from baseline in the collagen cross-link ratio in women receiving risendronate for 3 or 5 yr. Compared with placebo, however, the FTIRI cross-link ratio was significantly decreased, suggesting

lower collagen maturation with risendronate at the bone-forming sites. Conversely, at the bone-resorbing sites, there was no significant change in this enzymatic cross-link ratio after 3 or 5 yr of risendronate compared with baseline or placebo. Our data showing that the urinary α/β CTX ratio, which may reflect maturation of collagen at the bone-resorbing sites, did not significantly change after 1 yr of alendronate (and only slightly increase after 2 yr) are in agreement with the FTIRI data obtained with a different bisphosphonate on a different index of collagen maturation. More recently the effects of alendronate, ibandronate, estradiol, and raloxifene on urinary α/β CTX ratio have been studied in postmenopausal women participating in several trials, although they were not head to head trials.⁽³⁴⁾ It was found that alendronate and ibandronate, but not estradiol or raloxifene, produced a decrease in the urinary α/β CTX ratio that was significant compared with placebo within 6 mo of treatment and maintained over 2 yr. These data are not in agreement with the absence of a significant decrease of α/β CTX ratio that we observed with alendronate in our study. The reasons for these differences are unclear. In the study of Byrjalsen et al.,⁽³³⁾ women receiving alendronate were early non-osteoporotic postmenopausal women on average 18 yr younger than the osteoporotic subjects of the PaTH study. Among the 27 subjects treated with alendronate in this previous study,⁽³³⁾ 13 (48%) received the higher dose of 20 mg/d, resulting in a greater reduction of overall bone resorption (−91% versus −70% in PaTH of decrease of urinary α CTX/Cr after 12 mo). A single dose of intravenous zoledronic acid (200 or 400 μ g) given to patients with Paget's disease, characterized initially by a 3-fold higher α/β CTX ratio than age-matched controls, also reduced the urinary α/β CTX ratio.⁽³⁴⁾ It may thus be possible that profound suppression of bone turnover and/or high pretreatment of the α/β CTX ratio is required to induce detectable changes in the urinary α/β CTX ratio with bisphosphonate therapy.

In vitro experiments using synthetic α CTX peptide or fetal trabecular mineralized bone collagen powder indicated that the equilibrium of the isomerization reaction is achieved after ~300 days at 37°C.⁽²⁵⁾ These in vitro studies also predicted that, at equilibrium, the α/β CTX ratio is 0.25.⁽²⁵⁾ This equilibrium ratio is actually very close to the mean urinary α/β CTX ratio (0.23) we observed in healthy premenopausal women. Thus, in bone tissue of healthy young women who do not fracture, on average, isomerization of type I collagen molecules has reached an equilibrium state. At baseline, in untreated postmenopausal women of the PaTH study, the urinary α/β CTX ratio was significantly higher than in premenopausal controls. This suggests that, on average, in postmenopausal women, the extent of isomerization of collagen molecules is moderately decreased, possibly because the increased in bone turnover does not allow the kinetic of CTX isomerization to reach equilibrium. After 1 yr of PTH followed by 1 yr of alendronate or placebo, both the average α/β CTX ratio and its variability (SD: 40% versus 19% of the mean value in pre- and postmenopausal women after PTH treatment, respectively) further increased. Thus, it seems that in some postmenopausal women with osteoporosis,

PTH treatment could be associated with a lower extent of type I collagen isomerization. The consequences of this biochemical change in type I collagen on bone fracture resistance remains unclear. In human vertebral bodies, there was a negative association between the $\alpha\alpha/\beta\beta$ CTX ratio directly measured in bone tissue extract and compressive mechanical fracture resistance independent of BMD.⁽²²⁾ In untreated postmenopausal women, urinary $\alpha\alpha/\beta\beta$ CTX in the highest quartile was associated with a 2-fold increased risk of incident fracture, which remained significant for nonvertebral fractures after adjustment for BMD and overall bone turnover evaluated by serum bone alkaline phosphatase.⁽²⁸⁾ Thus, these studies suggest that an abnormally high $\alpha\alpha/\beta\beta$ CTX ratio could be associated with lower bone fracture resistance. However, translating these previous data to the findings of this study should be done cautiously. Indeed, it is likely that the association between urinary $\alpha\alpha/\beta\beta$ CTX ratio and altered fracture resistance is not linear but may follow a U-shape pattern⁽³⁵⁾ and could be observed only when this ratio exceeds a threshold whose value remains to be determined. In addition, the relationships between the $\alpha\alpha/\beta\beta$ CTX ratio and fracture risk observed in untreated postmenopausal women may not directly apply to subjects receiving PTH or alendronate. As reported in the original PaTH study papers,^(10,29) because of the small sample size, the effect of the different treatment regimens on fracture incidence and consequently its relationship with changes in the urinary $\alpha\alpha/\beta\beta$ CTX could not be assessed.

Histological studies have estimated that, in healthy adults, bone tissue may rest on average for ~2.5 yr before being remodeled.⁽²³⁾ Thus, in healthy adults, on average, it is expected that the isomerization of type I collagen will reach almost equilibrium before bone matrix is being degraded and reflected by changes in the urinary ratio of C-telopeptide degradation products. Conversely, in pathological situations including patients with Paget's disease of bone, because of a marked increase of bone remodeling in localized skeletal area, the equilibrium of the isomerization reaction is not achieved before the newly formed bone matrix is being degraded. The degradation of this incompletely isomerized collagen is reflected by a marked increase of the urinary $\alpha\alpha/\beta\beta$ CTX ratio in these patients.^(22,24)

Our study has both strengths and limitations. This is the first study reporting the effects of PTH(1-84) and alendronate in a randomized clinical trial on a noninvasive biological index that is supposed to reflect bone collagen maturation. Because the PaTH study did not include a placebo group, we can not exclude that alendronate or PTH(1-84) may induce changes in the urinary $\alpha\alpha/\beta\beta$ CTX ratio compared with physiological age-related changes. However, cross-sectional studies indicate that, in untreated postmenopausal women, the $\alpha\alpha/\beta\beta$ CTX ratio remains fairly stable with age.^(27,33) We can also not exclude that longer treatment with PTH(1-84) or alendronate would result in changes in this isomerization ratio. Finally, these data may not translate to teriparatide and to bisphosphonates other than alendronate.

In summary, we found that 1 yr of PTH(1-84) followed by 1 yr of placebo or alendronate was associated with an increased urinary $\alpha\alpha/\beta\beta$ CTX ratio in postmenopausal

women with osteoporosis. These data suggest that treatment with PTH(1-84) could be associated, after a certain delay, with decreased type I collagen isomerization. This may be indicative of a reduction in mean tissue age, possibly related to an increase in bone remodeling. The influence of these biochemical changes in type I collagen isomerization with PTH(1-84) on bone fracture resistance remains to be studied.

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Address reprint requests to:

Patrick Garnero, PhD

Synarc

16 rue Montbrillant

69003 Lyon, France

E-mail: patrick.garnero@synarc.com

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