

Raloxifene and Estrogen: Comparative Bone-Remodeling Kinetics*

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ABSTRACT

The pattern of changes in human bone remodeling produced by raloxifene (60 mg/day) was compared to that of estrogen (given as hormone replacement therapy) in 33 early postmenopausal women randomly assigned to raloxifene, estrogen, or no treatment. Remodeling was measured using calcium tracer kinetic methods employed under a constant diet and full metabolic balance conditions. Studies were performed at baseline and, to detect both early and late remodeling changes, at 4 and 31 weeks of treatment. Both raloxifene and estrogen produced a significant positive calcium balance shift at each treatment measurement point: +74 and +60 mg/day at 4 weeks, and +60 and +91 mg/day at 31 weeks for raloxifene and estrogen, respectively. Externally, this balance change was due to a highly significant fall in the urinary calcium level

and marginal improvement in calcium absorption efficiency. Internally, bone resorption was significantly reduced at both measurement points: -64 and -60 mg/day at 4 weeks, and -82 and -162 mg/day at 31 weeks for raloxifene and estrogen, respectively. Bone formation was not significantly affected by either agent at 4 weeks; at 31 weeks, formation was reduced by estrogen, but not by raloxifene. Thus, at 4 weeks, the general pattern of remodeling change was identical for the two agents. At 31 weeks, remodeling suppression was greater for estrogen than for raloxifene; however, remodeling balance was the same for the two agents. We conclude that raloxifene and estrogen affect the bone remodeling apparatus similarly, and that raloxifene, therefore, is acting on bone as an estrogen agonist. (*J Clin Endocrinol Metab* 82: 3425-3429, 1997)

SELECTIVE estrogen receptor modulators (SERMs) are a set of chemically diverse compounds that bind and interact with the estrogen receptor, but which in certain tissues act as estrogen agonists and in others as estrogen antagonists (1). Raloxifene, a SERM without estrogen agonist effects on the endometrium, has been reported to suppress biochemical markers of bone remodeling, much as does estrogen (2, 3). However, the biochemical markers of remodeling have not quantitatively been calibrated against actual remodeling as measured by total body calcium kinetics. The uncertainty surrounding interpretation of remodeling marker changes after various treatments is highlighted by the fact that the biomarkers differ in response from marker to marker. Presumably, they reflect changes in bone cell biology that are not strictly parallel to volumetric bone resorption or formation. Accordingly, it seemed important to assess the bone-remodeling effects of raloxifene directly, using established calcium kinetic methods. We report here comparative results of estrogen and raloxifene on the components of bone-remodeling activity in early postmenopausal women, the principal population for which this agent is likely to be used. Our purpose was mainly exploratory, *i.e.* to determine whether the pattern of change in remodeling over time was the same for the two agents.

Materials and Methods

Design

The study employed a randomized, open design, that compared no treatment, raloxifene hydrochloride in a daily dose of 60 mg, and es-

trogen given as cyclic hormone replacement therapy (0.625 mg Premarin daily with 5 mg medroxyprogesterone daily for the first 2 weeks of each month of treatment). The plan is shown diagrammatically in Fig. 1. Studies were performed at baseline, after 4 weeks of treatment with active agent, and after 30-32 weeks of treatment (achieved intervals were 4.37 ± 0.42 weeks for the early and 31.23 ± 2.91 weeks for the late phase studies, respectively). Study timing was based on current understanding of the effects of estrogen on bone remodeling biology (4, 5). Estrogen is believed to suppress the activation step of the remodeling process and thus to have its initial effect on bone resorption, followed several weeks later by a corresponding reduction in bone formation. It was expected, therefore, that the 4-week treatment study would detect a reduction in resorption (if it had occurred), but find little change in formation, while the 30- to 32-week interval, being longer than one bone remodeling period (*i.e.* one sigma), would measure the new steady state, with no further reduction in resorption, but a reduction in formation.

Subjects

Subjects were women from 2-8 yr postmenopause who had either never received postmenopausal estrogen replacement therapy or who had had no estrogen for at least 24 months. For entry, serum estradiol had to be below 20 pg/mL. Subjects were in good health, of stable weight, nonsmokers, not currently or recently taking medications known to affect bone remodeling, and with no fractures for at least the past year. Pertinent subject characteristics are as set forth in Table 1. There were no significant differences among the three groups. The protocol had been approved by the Creighton University institutional review board, and each woman gave written informed consent.

The target group sizes were 12 each in the active treatment arms and six in the no treatment group. Because of anticipated intercurrent events over the course of a study of this duration, we overrecruited by ~20%. As it turned out we lost 1 of 7 women randomized to the control group because of a traumatic hip fracture occurring after the baseline study and 1 from the raloxifene group when we learned that she had an occult eating disorder (which would have disqualified her on screening). Additionally, two subjects in the control group developed uncomplicated extremity fractures (ankle and wrist, respectively) just before the scheduled second in-patient study. Accordingly, the middle study for these 2 subjects was aborted because of the possibility that the fracture-healing process and/or the changed mobility would perturb total body remod-

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eling kinetics sufficiently to render comparisons with the active treatment arms invalid. Additionally, total serum alkaline phosphatase in these 2 subjects was monitored serially, and although elevation above baseline values was minimal, the final kinetics study in both subjects were not performed until their alkaline phosphatase values had fully returned to baseline levels.

Protocol

All studies were performed while subjects were in-patients on a metabolic unit. The women ingested a single daily diet matched to habitual calcium intake, as determined from analysis of individual 7-day diet diaries obtained before admission. Diets were gelatin free and low in collagen. Duplicate diets were prepared for each patient visit and chemically analyzed for calcium, as described below. All medications that subjects were taking before admission (including vitamin and mineral supplements) were continued throughout the in-patient study. Each was analyzed for its calcium content, which was included as a part of the total calcium intake. Subjects were encouraged not to change their habitual dietary or supplement-taking practices over the 1-yr course of the study.

Complete collections of excreta were obtained throughout each 9-day in-patient stay. Urine was collected in 8-h pools for 3 days after tracer injection (see below) and in 24-h pools at all other times. Feces were collected in two 4-day pools. During the in-patient stay, 1.0 g polyethylene glycol (PEG; Carbowax 4000, Fisher Scientific, Fairlong, NJ) was given with each meal or major snack. PEG recovery in stool specimens was used to define the intake interval (both timing and duration) to which a given fecal collection corresponded. In this way PEG recovery was used both to quantify daily fecal calcium excretion and to time fecal isotope excretion for calculation of endogenous fecal calcium loss. This is a step of critical importance in studies in women of this age ingesting generally low fiber diets. Dawson-Hughes *et al.* (6) had previously shown that it takes up to 12 days to clear 95% of a nonabsorbable marker

from the colon of such women, and in this study, the measured fecal lag averaged 74 h, with the maximum being 165 h.

On the first morning of the in-patient study, before beginning the balance and kinetic collections, a 2-h urine sample was collected for measurement of the fasting hydroxyproline to creatinine ratio (Hypro:Cr); on the ninth morning, an oral calcium absorption test was performed (see below). Routine serum chemistry panels and serum lipid levels were obtained at baseline and 1, 4, and 7 months after starting therapy.

To minimize the level of residual radioactivity (from the baseline study) at the time of the 4-week treatment study, the second study was scheduled to occur approximately 6 months after the first. Accordingly, treatment with active agent was scheduled to start at about the 5-month point after the baseline study. Thus, the entire study in each woman lasted about 1 yr (Fig. 1). Studies in the untreated individuals were scheduled to match the timing of the studies in the treated subjects and also extended over approximately 1 yr.

Calcium kinetic analysis

Bone remodeling variables (mineral resorption and mineral accretion, approximately equivalent to volumetric bone resorption and bone formation, respectively) were calculated from pool calcium specific radioactivity dilution over time. Details of the theory (7) and methods (8) have been set forth elsewhere. Briefly, ^{45}Ca was given iv as high specific activity $^{45}\text{CaCl}_2$ in 5.0 mL saline at 1000 h on day 2 of the in-patient study in a dose of approximately 7 μCi (0.26 megabecquerels). Frequent blood samples at carefully noted times were obtained throughout the remainder of the 9-day stay and were analyzed for their stable and radioactive calcium contents. Serum calcium specific radioactivity values, expressed as a fraction of the administered tracer dose per g calcium, were combined with corresponding values from the urine pools and plotted against time. The resulting curves were fitted to a three-term exponential equation using PK Analyst Software (MicroMath, Salt Lake City, UT). Fits in all cases were extremely good, with $r^2 > 0.99$ in every instance. The equation parameters derived from the fitting process were used both to compute the time integral of the specific activity curve for calculation of endogenous fecal calcium excretion rate (9) and as input to the equations for pool size, pool turnover, bone resorption, and bone mineral accretion (7). The primary measurement datum with this approach is pool turnover (expressed here in milligrams of Ca per day). Bone mineral accretion and resorption are derived from turnover by difference, *i.e.* accretion = turnover - urinary Ca - endogenous fecal Ca (Eq I) and resorption = turnover - absorbed Ca (Eq II).

Endogenous fecal calcium was measured by the clearance of the iv tracer through the gut (9), and absorbed calcium was calculated as the product of calcium intake and measured absorption fraction (see below), adjusting the latter for the known effects of load size on absorption efficiency (10).

Fractional calcium absorption was measured on day 9 of each in-patient study after the collections needed for remodeling kinetic analysis had been completed. The procedure used a single $\sim 10\text{-}\mu\text{Ci}$ oral dose of ^{45}Ca in a carrier of 200 mg calcium (as fortified orange juice) ingested as a part of a light breakfast and timed at a point exactly 2 h earlier in the day than the iv dose given 7 days earlier. A single blood sample was taken exactly 5 h after the oral dose, and absorption was calculated from the increment in serum ^{45}Ca specific radioactivity over the level measured just before administration of the oral dose. This calculation has been described previously (11, 12) and amounted simply to a suitable correction of the measured serum level for differing volumes of distri-

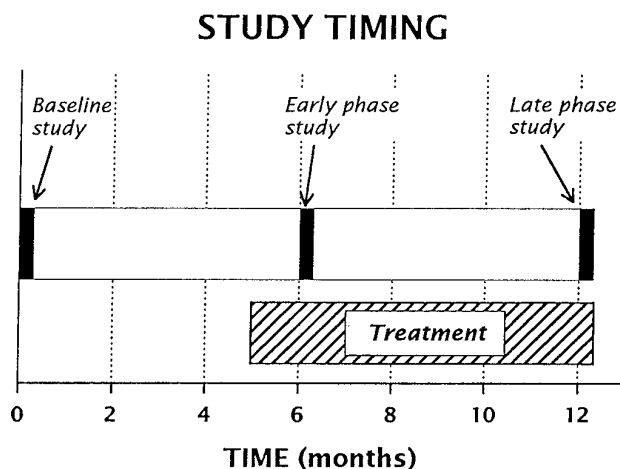


FIG. 1. Schematic overview of project, showing the times of the three balance-kinetic studies relative to duration and onset of active treatment. (Copyright Robert P. Heaney, 1997; reproduced with permission.)

TABLE 1. Subject characteristics by treatment group^a

	Estrogen	Raloxifene	No treatment
N	14	13	6
Height (m)	1.650 ± 0.064	1.638 ± 0.064	1.644 ± 0.029
Weight (kg)	66.9 ± 10.3	69.7 ± 9.6	71.1 ± 3.6
BMI (kg/m ²)	24.5 ± 2.7	25.9 ± 2.2	26.3 ± 1.3
Age	54.9 ± 3.4	54.7 ± 4.2	53.5 ± 2.7
Years postmenopause	4.3 ± 1.6	4.7 ± 1.8	4.8 ± 2.3
Calcium intake (g)	1.182 ± 0.582	0.892 ± 0.475	1.332 ± 0.962

^a Mean ± SD.

bution based on a woman's height and weight. Absorption was also calculated from the quotient of the serum specific activities at the 5 h postabsorption point and the 3 h value obtained 7 days earlier after the iv dose, using essentially the double tracer method of deGrazia *et al.* (13). For the purposes of this study, absorption fraction was taken as the average of the two estimates derived from the foregoing calculations.

Calcium balance was calculated as described previously (14). In this case, balance was based on the tracer measurements, which are more precise than traditional mass balances over short study periods (15), and can be summarized by the following equation: Ca balance = absorbed Ca - endogenous fecal Ca - urinary Ca (Eq III).

Analytical methods

Calcium in serum, diets, and excreta was measured by atomic absorption spectrophotometry. Urine and serum samples were analyzed directly. Paired duplicate diets and complete fecal specimens were freeze-dried and milled, and weighed aliquots were ashed at 600 C. The resulting ash was dissolved in hydrochloric acid for the measurement of calcium. Hydroxyproline was measured in urine by the method of Bergman and Loxley (16), creatinine by an automated system using the Gilford analyzer (Gilford Systems, Oberlin, OH) and the Jaffe method (17), and PEG in fecal pools by the method of Malawer and Powell (18). ^{45}Ca was measured by liquid scintillation counting in a Beckman LS-3150T counter (Beckman Instruments, Irvine, CA) against suitably diluted dose standards, water blanks (for radiation background), and blanks containing potassium solutions. (The latter were used for correction of the urine counts for the natural ^{40}K present in all potassium. Such corrections are essential in studies of this sort, as the calcium tracer doses are small, and as much as 5–20% of total urine counts may be derived from ^{40}K . Corrections were based on the individually analyzed potassium content of each urine sample.) Counts obtained during second and third studies and during absorption tests were also corrected for residual serum radioactivity from earlier tracer treatment.

Statistical analyses

Baseline values are reported as the mean \pm SD and are compared by simple one-way ANOVA across the three groups. Changes from baseline for each variable of interest were computed for both the 4 week and the 31 week measurement points, were reported as mean differences \pm SEM, and were tested against a null hypothesis of zero change. As the purpose of the study was not to determine the efficacy of either agent, but to see whether the time patterns of remodeling change were similar for the two agents, the major comparisons were between the raloxifene and estrogen groups. (The no treatment group was included mainly to

ensure that we had not overlooked unexpected changes occurring over the approximately 1 yr of the study in either bone biology or analytical methods.) Comparisons between the within-subject changes in the raloxifene and estrogen groups were made using *t* tests of means.

Results

Table 2 presents the baseline values for the principal balance and kinetic variables, and Tables 3 and 4 show the changes from baseline at the early and late measurement points. The baseline values were generally typical of those found in early postmenopausal women and are presented here mainly to serve as a referent for interpretation of the changes induced by treatment. As expected for women at this life stage, calcium balance was negative (mean over all three groups, -66 mg/day; SEM, 14; for $H_0 = 0$, $P < 0.001$).

At the early measuring point (Table 3), urinary calcium and bone resorption were significantly reduced in both the estrogen- and raloxifene-treated groups. Additionally, pool turnover was significantly reduced in the estrogen-treated group, but only marginally so in the raloxifene-treated group. No significant change in these variables occurred in the no treatment group. The reduction in resorption amounted to about 15% of the baseline values for both estrogen and raloxifene. Calcium balance shifted positively in both the raloxifene and estrogen groups by amounts sufficient to obliterate the negative balance seen at baseline. This change was reflected mainly in the fall in urinary calcium, which was reduced by about 25% in both active treatment groups. There was, as well, a nonsignificant increase in intestinal absorption efficiency, making the balance shift larger than the fall in urinary calcium. There was no statistically significant difference between the effects of the two agents on any of the remodeling or balance variables. As had been predicted, bone mineral accretion was not altered by either agent at this early time point.

At the late measuring point (Table 4), urinary calcium, pool turnover, and bone resorption all were significantly reduced from baseline in both the estrogen- and raloxifene-

TABLE 2. Baseline values for calcium balance and kinetics^a

Variable	Estrogen	Raloxifene	No treatment
Urine Ca (mg/d)	200 \pm 102	153 \pm 53	177 \pm 83
Absorption fraction	0.292 \pm 0.061	0.292 \pm 0.076	0.266 \pm 0.070
Balance (mg/d)	-64 \pm 79	-68 \pm 71	-82 \pm 112
Pool turnover (mg/d)	672 \pm 115	623 \pm 106	668 \pm 117
Resorption (mg/d)	417 \pm 114	400 \pm 119	439 \pm 85
Accretion (mg/d)	351 \pm 93.2	332 \pm 109	355 \pm 127
Hypro/creatinine ($\mu\text{mol}/\text{mmol}$)	21.0 \pm 5.92	20.7 \pm 4.04	18.7 \pm 5.24

^a Mean \pm SD.

TABLE 3. Balance and kinetic changes from baseline at four weeks^a

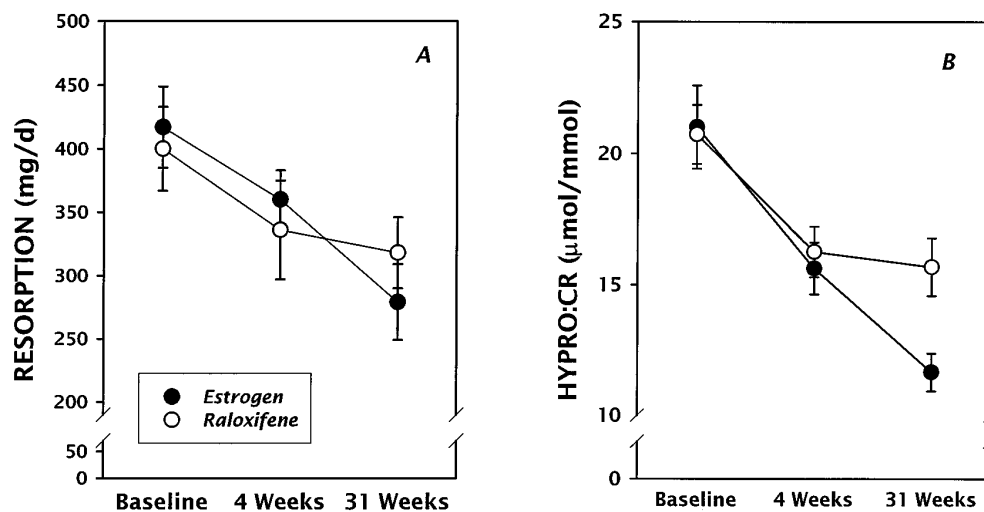
Variable	Estrogen	Raloxifene	No Treatment
Urine Ca (mg/d)	-47 \pm 8 ^b	-41 \pm 6 ^b	-21 \pm 40
Absorption fraction	+0.013 \pm 0.015	+0.018 \pm 0.020	-0.006 \pm 0.021
Balance (mg/d)	+57 \pm 25 ^b	+74 \pm 18 ^b	+10 \pm 45
Pool turnover (mg/d)	-57 \pm 14 ^b	-37 \pm 18	-20 \pm 45
Resorption (mg/d)	-57 \pm 17 ^b	-64 \pm 23 ^b	-1 \pm 55
Accretion (mg/d)	-12 \pm 20	+10 \pm 23	+9 \pm 57
Hypro/creatinine ($\mu\text{mol}/\text{mmol}$)	-5.4 \pm 1.5 ^b	-4.8 \pm 1.1 ^b	-3.0 \pm 1.3

^a Mean \pm SE.

^b $P < 0.05$ with respect to zero change.

TABLE 4. Balance and kinetic changes from baseline at 31 weeks^a

Variable	Estrogen	Raloxifene	No Treatment
Urine Ca (mg/d)	-43 ± 7 ^b	-38 ± 6 ^b	-4 ± 19
Absorption fraction	+0.036 ± 0.019	+0.014 ± 0.016	-0.027 ± 0.029
Balance (mg/d)	+68 ± 29 ^b	+60 ± 17 ^b	-33 ± 46
Pool turnover (mg/d)	-112 ± 19 ^b	-68 ± 15 ^b	-7 ± 21
Resorption (mg/d)	-137 ± 37 ^b	-82 ± 18 ^b	+26 ± 59
Accretion (mg/d)	-70 ± 19 ^b	-22 ± 21	-16 ± 21
Hypro/creatinine (μmol/mmol)	-9.4 ± 1.5 ^b	-5.1 ± 0.8 ^b	-0.6 ± 1.1

^a Mean ± SE.^b *P* < 0.05 with respect to zero change.**FIG. 2.** Pattern of change over time in bone resorption-related variables. A, Mineral resorption. B, Hypro:Cr. The error bars in both panels are 1 SEM. (Copyright Robert P. Heaney, 1997; reproduced with permission.)

treated groups. None of the bone remodeling variables changed significantly in the no treatment group. In the estrogen group, there was a further significant fall in resorption relative to that in the early phase study, whereas there was no further change in the raloxifene group. Bone mineral accretion was significantly reduced at this late time point with estrogen, but not with raloxifene. As at 4 weeks, calcium balance was positively shifted from the baseline. There were no significant changes in urinary calcium, absorption fraction, or balance in either treatment group in the interval from 4–31 weeks, *i.e.* all of the balance effect was achieved by 4 weeks; it was simply maintained thereafter.

One woman randomized to raloxifene turned out to have had an unusually low baseline accretion rate (123 mg/day). She actually had an increase in accretion rate on raloxifene (at both 4 and 31 weeks), but to levels comparable to the treatment-associated lowered levels of the other subjects. Because of the relatively small group size, her presence could have been the reason for the absence of statistical significance for the downward shift in accretion in the raloxifene group as a whole. Accordingly, we reanalyzed the changes in the raloxifene-treated group with her values excluded (results not shown). Eliminating her values did not change any of the basic findings, except that the 4-week drop in pool turnover became statistically significant. Moreover, the 31-week change in bone formation remained not different from zero.

The only biochemical marker of remodeling that we measured was Hypro:Cr. Hypro:Cr fell at the 4 week measure-

ment point (Table 3), in parallel with the kinetic resorption rate. It continued to fall at the 31 week point in the estrogen-treated group, but not in the raloxifene-treated women, in whom it remained essentially unchanged from its 4 week value. The parallelism of the changes in kinetic resorption and Hypro:Cr is shown graphically in Fig. 2.

Both estrogen and raloxifene significantly reduced serum total calcium, albumin, and low density lipoprotein cholesterol (by amounts in the range of 4–10%). Total alkaline phosphatase was slightly, but nonsignificantly, reduced by both agents.

Discussion

We have shown here that raloxifene, like estrogen, reduces bone resorption without significantly altering bone formation over the short term. This sequence of effects is consistent with the basic multicellular unit concept of Frost (4). The external manifestation of this change was a positive shift in calcium balance. Four weeks after starting therapy, the skeletal effects of the two agents were qualitatively and quantitatively indistinguishable. Changes in serum calcium, albumin, and low density lipoprotein cholesterol were also similar for the two agents. We conclude, therefore, that the short term effects of raloxifene on the bone-remodeling apparatus are consistent with activity as an estrogen agonist.

However, there appeared to be some divergence between the remodeling effects of the two agents at the 31 week

measurement point. For both agents, calcium balance did not change further, and urinary calcium and intestinal absorption remained at their 4 week level. However, with estrogen, both resorption and formation were reduced further and by approximately the same extent (hence the absence of a significant additional balance change). With raloxifene, neither resorption nor formation changed significantly at 31 weeks from their 4 week levels.

Although it is generally accepted that estrogen is a remodeling suppressor (5, 19), we know of no carefully timed experiments that have been performed that could test concordance between its actual effects and the sequence of changes predicted to be produced by remodeling suppression (4). Thus, this investigation not only compared estrogen and raloxifene, but also tested some of the predictions of existing bone remodeling theory. The 4-week fall in resorption without a change in formation and the later fall in accretion produced by estrogen constitute a pattern consistent with what existing theory predicts for activation suppression. However, the continuing fall in resorption produced by estrogen between 4–31 weeks had not been expected. If the action of estrogen is to influence the threshold for activation of remodeling loci, it is not clear why activation would continue to drop after the new estrogen status of the body has been established. Possibly, the generation time of the cell lines concerned is longer than 4 weeks.

Although there were no significant differences between estrogen and raloxifene in any of the changes from baseline at either the 4 or 31 week measurement points, the changes in turnover, resorption, and accretion occurring from 4–31 weeks in the estrogen group were significantly different from zero, but were not for raloxifene, and this remained true even when the raloxifene-treated woman with low basal turnover was excluded from the analysis. Perhaps of more interest, there was no significant suppression of formation with raloxifene. We do not believe that this difference is simply a matter of relative potency. Evans *et al.* (20) have reported that estrogen suppresses cancellous bone-forming activity in an ovariectomized rat model, whereas raloxifene does not; the other bony effects of the two agents were similar. As Fig. 2 shows graphically, the falls in resorption were essentially identical for the two agents at the 4 week measurement point. It seems likely that there is some qualitative difference in the mode of action of the two agents. This suggestion is supported by the finding of Guinness *et al.* (21) that the effects of estrogen on serum levels of the insulin-like growth factors are suppressive, whereas with raloxifene, insulin-like growth factor levels rise. These subtle differences underscore the fact that SERMs are, after all, selective, and in each case their profiles of tissue activity need to be determined.

Although we did not set out to test the validity or usefulness of the biochemical markers of remodeling, it is nevertheless interesting to note the similarity in the pattern of

responses of Hypo:Cr and kinetically measured resorption rate. Hypo:Cr even detected the divergence of effects of raloxifene and estrogen from 4–31 weeks. It seems that when adequate care is taken, this, the oldest of the resorption markers, can be useful and reliable.

In conclusion, raloxifene, like estrogen, reduced bone remodeling in estrogen-deficient early postmenopausal women and induced a positive calcium balance shift maintained out to 31 weeks of treatment. Except for the absence of suppression of bone mineral accretion, raloxifene seems to act on the bone remodeling apparatus as an estrogen agonist.

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