

Table D3 Iliac Crest Biopsy Data (Mean  $\pm$  SEM of Raw Data) at 15 Months (n = 121)

	Sham	OVX	PTH1	PTH1-W*	PTH5	PTH5-W*
Lb.Ar	26.8 $\pm$ 1.7 (21)	22.3 $\pm$ 1.9 (20)	38.4 $\pm$ 2.5 (19)	31.2 $\pm$ 1.9 (19)	48.5 $\pm$ 2.5 (21)	33.0 $\pm$ 2.5 (19)
Tb.Th	147 $\pm$ 7 (21)	137 $\pm$ 7 (20)	159 $\pm$ 7 (19)	157 $\pm$ 8 (20)	190 $\pm$ 13 (21)	152 $\pm$ 11 (19)
Tb.N	1.81 $\pm$ 0.07 (21)	1.59 $\pm$ 0.09 (20)	2.41 $\pm$ 0.12 (19)	2.00 $\pm$ 0.09 (20)	2.64 $\pm$ 0.13 (21)	2.18 $\pm$ 0.09 (19)
Tb.Sc	422 $\pm$ 24 (21)	540 $\pm$ 51 (20)	279 $\pm$ 29 (19)	367 $\pm$ 29 (20)	207 $\pm$ 16 (21)	319 $\pm$ 20 (19)
E.Pm	1.92 $\pm$ 0.44 (21)	4.12 $\pm$ 0.78 (20)	4.40 $\pm$ 0.48 (19)	4.32 $\pm$ 0.73 (20)	4.64 $\pm$ 0.64 (21)	3.65 $\pm$ 0.46 (19)
Oc.Pm	0.66 $\pm$ 0.17 (21)	1.31 $\pm$ 0.25 (20)	1.66 $\pm$ 0.22 (19)	1.50 $\pm$ 0.28 (20)	1.48 $\pm$ 0.29 (21)	1.15 $\pm$ 0.23 (19)
O.Ar	0.65 $\pm$ 0.14 (21)	0.87 $\pm$ 0.11 (20)	1.96 $\pm$ 0.23 (19)	1.08 $\pm$ 0.15 (20)	1.81 $\pm$ 0.15 (21)	1.09 $\pm$ 0.12 (19)
O.Pm	23.9 $\pm$ 3.0 (21)	35.4 $\pm$ 3.0 (20)	46.1 $\pm$ 2.5 (19)	34.9 $\pm$ 3.7 (20)	48.1 $\pm$ 2.4 (21)	36.4 $\pm$ 3.2 (19)
Ob.Pm	2.09 $\pm$ 0.46 (21)	5.31 $\pm$ 1.02 (20)	9.82 $\pm$ 0.97 (19)	10.82 $\pm$ 1.68 (20)	9.56 $\pm$ 1.85 (21)	9.92 $\pm$ 1.44 (19)
L.O.Pm	56.2 $\pm$ 6.3 (21)	64.6 $\pm$ 5.0 (20)	65.9 $\pm$ 4.7 (19)	63.6 $\pm$ 4.4 (20)	74.8 $\pm$ 3.2 (21)	64.7 $\pm$ 5.0 (19)
Ms.Pm	12.7 $\pm$ 1.8 (21)	22.8 $\pm$ 2.4 (20)	29.9 $\pm$ 2.3 (19)	22.3 $\pm$ 2.4 (20)	35.9 $\pm$ 2.2 (21)	24.1 $\pm$ 2.9 (19)
MAR	0.72 $\pm$ 0.03 (21)	0.77 $\pm$ 0.03 (20)	0.75 $\pm$ 0.03 (18)	0.96 $\pm$ 0.05 (19)	0.81 $\pm$ 0.03 (21)	0.88 $\pm$ 0.05 (19)
Aj.AR	0.41 $\pm$ 0.05 (21)	0.50 $\pm$ 0.05 (20)	0.51 $\pm$ 0.04 (18)	0.62 $\pm$ 0.07 (19)	0.61 $\pm$ 0.04 (21)	0.58 $\pm$ 0.06 (19)
Oms	12 $\pm$ 1 (21)	12 $\pm$ 1 (20)	15 $\pm$ 2 (18)	10 $\pm$ 1 (19)	11 $\pm$ 1 (21)	10 $\pm$ 1 (19)
BFR/BV	42.9 $\pm$ 6.6 (21)	78.0 $\pm$ 9.1 (20)	92.8 $\pm$ 9.3 (18)	93.5 $\pm$ 14.4 (19)	104.1 $\pm$ 11.3 (21)	93.4 $\pm$ 13.8 (19)
BFR/BS	3517 $\pm$ 544 (21)	6449 $\pm$ 812 (20)	8422 $\pm$ 740 (18)	7837 $\pm$ 1004 (19)	10705 $\pm$ 661 (21)	7983 $\pm$ 1139 (19)
BFR/TtBV	11.1 $\pm$ 1.9 (21)	17.1 $\pm$ 2.3 (20)	33.0 $\pm$ 3.2 (18)	26.9 $\pm$ 3.9 (19)	48.0 $\pm$ 4.9 (21)	28.2 $\pm$ 3.8 (19)
FP	208 $\pm$ 36 (21)	139 $\pm$ 10 (20)	145 $\pm$ 13 (18)	136 $\pm$ 15 (19)	121 $\pm$ 9 (21)	142 $\pm$ 19 (19)
Rs.P	19.5 $\pm$ 7.5 (21)	15.3 $\pm$ 2.4 (20)	13.3 $\pm$ 1.7 (18)	21.2 $\pm$ 5.6 (19)	11.4 $\pm$ 1.7 (21)	16.4 $\pm$ 3.8 (19)
Rm.P	227 $\pm$ 41 (21)	155 $\pm$ 11 (20)	158 $\pm$ 14 (18)	157 $\pm$ 18 (19)	152 $\pm$ 9 (21)	159 $\pm$ 21 (19)
Ac.F	2.48 $\pm$ 0.32 (21)	2.58 $\pm$ 0.18 (20)	2.62 $\pm$ 0.22 (18)	2.86 $\pm$ 0.33 (19)	3.08 $\pm$ 0.26 (21)	3.01 $\pm$ 0.43 (19)
W.wi	57.8 $\pm$ 2.6 (21)	62.2 $\pm$ 3.5 (20)	67.1 $\pm$ 2.9 (19)	71.0 $\pm$ 2.5 (20)	66.4 $\pm$ 3.0 (21)	65.5 $\pm$ 3.3 (19)
O.Th	8.02 $\pm$ 0.58 (21)	9.11 $\pm$ 0.51 (20)	10.59 $\pm$ 1.19 (19)	8.91 $\pm$ 0.47 (20)	8.56 $\pm$ 0.42 (21)	8.14 $\pm$ 0.38 (19)

Number of specimens per group are shown in parentheses.

\* Withdrawal data represent changes measured in monkeys treated for 12 months with LY333334 and then withdrawn from treatment for 3 months.

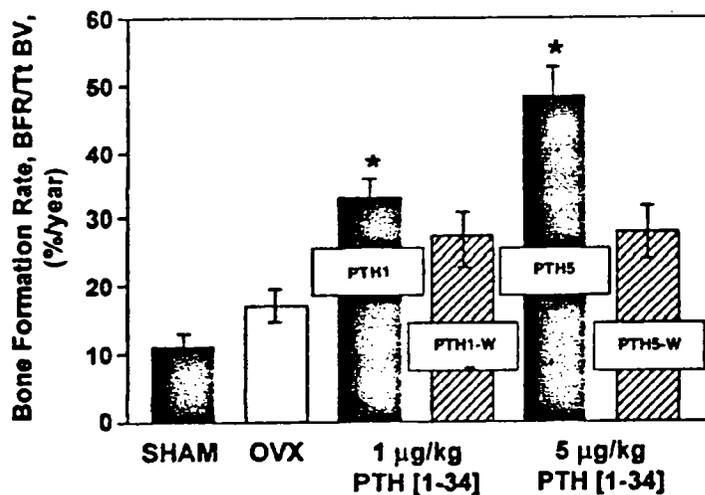


Figure 7.

Bone formation rate measured in iliac crest biopsies taken at 15 months from sham controls and ovariectomized adult monkeys, *M. fascicularis*, given vehicle or LY333334 at 1 µg/kg/day (PTH1) or 5 µg/kg/day (PTH5) for 18 months or at 1 µg/kg/day (PTH1-W) or 5 µg/kg/day (PTH5-W) for 12 months, after which treatment was withdrawn for a further 3 months. Note the increase in bone formation rates during active treatment in PTH1 and PTH5 and their reversion towards control values in PTH1-W and PTH5-W. Statistical significance, \*  $p < .05$ .

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Additional data on tunneling, cortical thickness in iliac crest:

**Table 1 Incidence of Tunneling in Iliac Crest Biopsies**

Treatment Duration	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
6 months	9.5 (21)	10.0 (20)	41.2 (17)	25.0 (20)	50.0 (20)	38.1 (21)
15 months	4.8 (21)	5.0 (20)	47.4 (19)	10.0 (20)	60.0 (20)	5.0 (20)

Number of specimens per group are shown in parentheses. Incidence in percent.

Conclusion: PTH caused a marked increase in iliac crest tunneling

**Table 4 Porosity and Cortical Thickness (Mean  $\pm$  SEM) of the Cortical Bone of the Iliac Crest Biopsies**

	Sham	OVX	PTH1	PTH1-W*	PTH5	PTH5-W*
<u>Porosity (%)</u>						
6 months	2.3 $\pm$ 0.3 (21)	2.8 $\pm$ 0.5 (18)	3.6 $\pm$ 0.7 (16)	3.3 $\pm$ 0.4 (19)	2.5 $\pm$ 0.5 (21)	2.7 $\pm$ 0.4 (17)
15 months	1.2 $\pm$ 0.2 <sup>b</sup> (19)	1.1 $\pm$ 0.1 <sup>b</sup> (18)	1.0 $\pm$ 0.1 <sup>c</sup> (16)	1.1 $\pm$ 0.2 <sup>c</sup> (17)	1.1 $\pm$ 0.2 <sup>c</sup> (17)	1.3 $\pm$ 0.3 <sup>c</sup> (14)
<u>Cortical Thickness (<math>\mu</math>m)</u>						
6 months	433.9 $\pm$ 11.7	403.2 $\pm$ 15.4	482.2 $\pm$ 22.6	452.0 $\pm$ 24.6	500.0 $\pm$ 28.1	399.3 $\pm$ 26.7
15 months	430.6 $\pm$ 17.7	446.6 $\pm$ 21.3	388.5 $\pm$ 20.3 <sup>b</sup>	398.7 $\pm$ 24.4	418.2 $\pm$ 33.1	440.6 $\pm$ 25.8

Number of specimens per group shown in parentheses.

\* Withdrawal data represent changes measured in monkeys treated for 12 months with LY333334 and then withdrawn from treatment for 3 months

<sup>b</sup> p < 0.05 vs. 6 months.

<sup>c</sup> p < 0.01 vs. 6 months.

Conclusion: PTH did not increase porosity in the iliac crest, unlike what was seen in the mid-humerus (see below, Table E4). At 6 months, cortical thickness was increased by PTH1 and PTH5, although the effect was not statistically significant. However, after 15 months of treatment the previously increased cortical thickness was decreased in both PTH1 and PTH5 groups to below- sham levels.

PTH did not affect the incidence of woven bone in specimens of the iliac crest at 15 months relative to 6 months.

These results indicate that it is unclear what the result of long term PTH treatment will be on a mixed bone site, like the iliac crest or the femoral neck, in terms of bone strength. However, histomorphometry (below, Table F16) and strength (above, Table B4) data for the femoral neck suggest that at this particular bone site adverse effects on bone density or strength do not occur.

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Humerus midshaft

Measurements were performed on periosteal, endocortical and intracortical surfaces of cortical bone from humerus mid-diaphysis

**Table E1** Definition of Histomorphometric Variables for Cortical Bone Measurements of the Humerus\*

Variable	Units	Description
Ac.F	cycles/year	Activation frequency
BFR.BS.Ec	$\mu\text{m}^3/\text{day}$	Bone formation rate, endocortical surface referent
BFR.BS.Ps	$\mu\text{m}^3/\text{day}$	Bone formation rate, periosteal surface referent
BFR.BV	%/year	Bone formation rate, bone volume referent
FP	days	Formation period
L.On.N/Ct.A	$\#/\text{mm}^2$	Number of fluorochrome labeled osteons per unit cortical area
MAR	$\mu\text{m}^3/\text{day}$	Mineral apposition rate, intracortical
MAR.Ec	$\mu\text{m}^3/\text{day}$	Mineral apposition rate, endocortical surface
MAR.Ps	$\mu\text{m}^3/\text{day}$	Mineral apposition rate, periosteal surface
MS/BS.Ec	%	Mineralizing endocortical surface normalized to total endocortical surface
MS/BS.Ps	%	Mineralizing periosteal surface normalized to total periosteal surface
O.Wi	$\mu\text{m}$	Osteoid width
Rs.N/Ct.A	$\#/\text{mm}^2$	Number of resorption spaces per unit cortical area
W.Wi	$\mu\text{m}$	Osteonal wall width
omt	days	Osteoid maturation time
Po	%	Porosity, the percentage of bone area occupied by spaces
B.Ar	$\text{mm}^2$	Bone area, the total area within the periosteal surface
Ct.Ar	$\text{mm}^2$	Cortical area, the area of bone within the periosteal surface (includes porosities)
Me.Ar	$\text{mm}^2$	Medullary cavity area

\* Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J Bone Miner Res* 2:595-610.

18 month data (Table E4):

Effect of OVX (relative to sham):

- Significant increases in Ac.F, BFR/BV, L.On.N/Ct.A, MS/BS.Ec, MS/BS.Ps, Rs.N/Ct.A, Po
- N.s. increase in BFR/BS.Ec, BFR/BS.Ps, O.Wi, Me.Ar
- No effect on Ct.Ar

Effect of LY treatment (relative to OVX):

- Significant increases in L.On.N/Ct.A, MS/BS.Ec (but not MS/BS.Ps), Rs.N/Ct.A, Po, Ct.Ar
- N.s. increases in Ac.F, BFR/BS.Ec\* (but not BFR/BS.Ps), BFR/BV\* (\*=only in PTH5 grp), B.Ar
- N.s. decrease in O.Wi, Me.Ar
- No effect on BFR/BS.Ps

Effect of LY treatment withdrawal:

- Significant decrease in Ac.F, BFR/BV, L.OnN/Ct.A, MS/BS.Ec, MS/BS.Ps (all but last of these parameters were increased by both OVX and LY, and were reversed below OVX levels, back to sham level, by LY withdrawal)
- Some other parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e. BFR/BS.Ec, BFR/BS.Ps, Rs.N/Ct.A)
- Decrease in porosity after withdrawal of PTH1 but not PTH5
- No effect on Ct.Ar or B.Ar

What this means:

Ovariectomy caused an increase in periosteal and endocortical bone formation and intracortical resorption, i.e. increased bone turnover. Ovariectomy caused increased porosity but had no effect on cortical bone area.

PTH5 but not PTH1 caused a further increase in endocortical but not periosteal bone formation. PTH caused an increase in cortical area apparently due to decreased medullary and increased bone area. PTH1 and PTH5 both increased cortical porosity. This appeared to be the result of an increase in intracortical resorption.

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The effect of PTH withdrawal was to decrease bone formation to below-OVX levels and occurred in both periosteal and endocortical zones. PTH1 withdrawal reversed the increased porosity. However, within the time frame studied, PTH5 withdrawal had no consequences for the treatment-induced effects on cortical area or porosity.

**Table E4** Intracortical Measurements (Mean  $\pm$  SEM of Raw Data) of the Humerus at 18 Months (n = 121)<sup>a</sup>

Variable	Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
Ac.F	1.85 $\pm$ 0.43 (19)	6.06 $\pm$ 0.76 (19)	7.69 $\pm$ 1.14 (19)	3.17 $\pm$ 0.49 (19)	9.08 $\pm$ 0.79 (21)	2.14 $\pm$ 0.32 (20)
BFR/BS.Ec	7.1 $\pm$ 2.2 (3)	20.9 $\pm$ 5.0 (15)	18.1 $\pm$ 3.3 (18)	14.9 $\pm$ 3.3 (10)	34.0 $\pm$ 4.3 (20)	13.9 $\pm$ 6.0 (8)
BFR/BS.Ps	3.8 $\pm$ 1.4 (5)	9.1 $\pm$ 2.0 (14)	8.5 $\pm$ 2.6 (17)	3.6 $\pm$ 1.1 (12)	9.0 $\pm$ 1.4 (17)	5.8 $\pm$ 2.0 (5)
BFR/BV	2.1 $\pm$ 0.5 (19)	9.2 $\pm$ 1.2 (19)	9.2 $\pm$ 1.4 (19)	4.5 $\pm$ 0.8 (19)	13.5 $\pm$ 1.2 (21)	2.3 $\pm$ 0.4 (20)
FP	82.7 $\pm$ 9.4 (19)	65.9 $\pm$ 4.5 (19)	63.4 $\pm$ 2.3 (19)	65.5 $\pm$ 2.8 (19)	61.7 $\pm$ 2.5 (21)	82.5 $\pm$ 10.3 (20)
L.Om.N/CLA	0.28 $\pm$ 0.06 (21)	1.03 $\pm$ 0.12 (19)	1.26 $\pm$ 0.16 (19)	0.52 $\pm$ 0.08 (20)	1.47 $\pm$ 0.10 (21)	0.40 $\pm$ 0.06 (20)
MAR	0.91 $\pm$ 0.08 (19)	1.07 $\pm$ 0.04 (19)	0.98 $\pm$ 0.03 (19)	1.04 $\pm$ 0.06 (19)	1.07 $\pm$ 0.02 (21)	0.88 $\pm$ 0.06 (20)
MAR.Ec	0.48 $\pm$ 0.11 (3)	0.75 $\pm$ 0.06 (15)	0.66 $\pm$ 0.04 (18)	0.66 $\pm$ 0.05 (10)	0.75 $\pm$ 0.03 (20)	0.61 $\pm$ 0.06 (8)
MAR.Ps	0.62 $\pm$ 0.11 (5)	0.69 $\pm$ 0.06 (14)	0.89 $\pm$ 0.23 (17)	0.54 $\pm$ 0.04 (12)	0.66 $\pm$ 0.04 (17)	0.83 $\pm$ 0.07 (5)
MS/BS.Lc	3.1 $\pm$ 1.4 (21)	21.0 $\pm$ 4.1 (19)	25.2 $\pm$ 3.9 (19)	12.0 $\pm$ 3.3 (20)	42.3 $\pm$ 5.2 (21)	9.2 $\pm$ 3.5 (20)
MS/BS.Ps	1.8 $\pm$ 0.8 (21)	10.0 $\pm$ 2.4 (19)	8.6 $\pm$ 1.3 (19)	4.0 $\pm$ 1.1 (20)	11.5 $\pm$ 2.1 (21)	2.0 $\pm$ 0.8 (20)
O.Wi	3.77 $\pm$ 0.20 (21)	4.04 $\pm$ 0.21 (19)	3.66 $\pm$ 0.15 (19)	4.03 $\pm$ 0.18 (20)	3.72 $\pm$ 0.11 (21)	3.75 $\pm$ 0.19 (20)
Rs.N/CLA	0.12 $\pm$ 0.04 (21)	0.21 $\pm$ 0.03 (19)	0.28 $\pm$ 0.04 (19)	0.12 $\pm$ 0.02 (20)	0.45 $\pm$ 0.05 (21)	0.17 $\pm$ 0.04 (20)
W.Wi	63.2 $\pm$ 3.0 (21)	68.6 $\pm$ 3.5 (19)	61.4 $\pm$ 1.8 (19)	66.0 $\pm$ 2.3 (19)	65.4 $\pm$ 2.1 (21)	63.0 $\pm$ 1.7 (20)
omt	4.58 $\pm$ 0.29 (19)	3.87 $\pm$ 0.24 (19)	3.76 $\pm$ 0.16 (19)	3.96 $\pm$ 0.14 (19)	3.5 $\pm$ 0.13 (21)	4.82 $\pm$ 0.55 (20)
Po	1.32 $\pm$ 0.13 (21)	2.61 $\pm$ 0.32 (19)	4.65 $\pm$ 1.10 (19)	2.31 $\pm$ 0.36 (20)	6.65 $\pm$ 0.94 (21)	6.46 $\pm$ 0.97 (20)
B.Ar	53.1 $\pm$ 1.2 (21)	52.8 $\pm$ 1.6 (19)	54.2 $\pm$ 1.4 (19)	55.3 $\pm$ 1.4 (20)	55.4 $\pm$ 1.3 (21)	57.9 $\pm$ 2.0 (20)
Ct.Ar	37.4 $\pm$ 0.8 (21)	35.4 $\pm$ 1.2 (19)	37.6 $\pm$ 0.9 (19)	38.1 $\pm$ 1.1 (20)	40.6 $\pm$ 0.9 (21)	40.8 $\pm$ 1.3 (20)
Mc.Ar	15.7 $\pm$ 0.9 (21)	17.5 $\pm$ 1.0 (19)	16.6 $\pm$ 0.9 (19)	17.3 $\pm$ 0.7 (20)	14.8 $\pm$ 1.1 (21)	17.1 $\pm$ 1.4 (20)

Number of specimens per group are shown in parentheses.

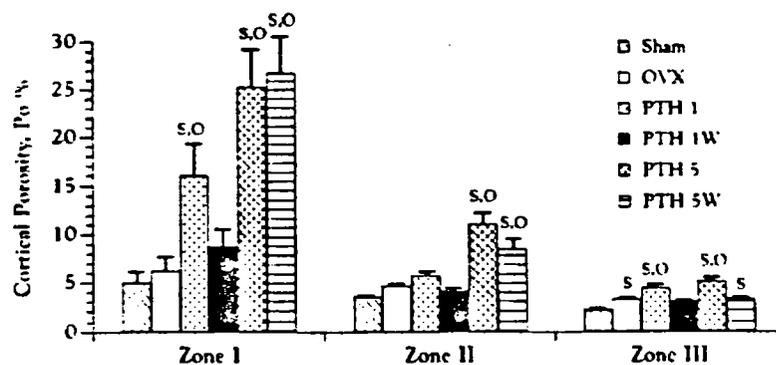
Although the total sample size was n = 121 monkeys, it was not always possible to measure each parameter due to label escape. Missing measurements also prevent calculation of derived variables, reducing the sample size for some variables.

<sup>a</sup> The total n for some variables is less than 121 due to missing values.

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Evaluation of three different cortical zones in the mid-humerus revealed that porosity was largest and/or most increased by LY treatment near the endocortical surface, and least near the periosteal surface (Fig. 10, below). Porosity in this endocortical zone was also largest to begin with (see Sham data).



**Figure 10.** Localization of porosities to predominantly the endocortical region of the midshaft humerus in sham controls and ovariectomized adult monkeys, *M. fascicularis*, given vehicle or LY333334 at 1 µg/kg/day (PTH1) or 5 µg/kg/day (PTH5) for 18 months or at 1 µg/kg/day (PTH1-W) or 5 µg/kg/day (PTH5-W) for 12 months, after which treatment was withdrawn for a further 6 months. (A) In the PTH-treated groups, porosity increased most near the endocortical surface (Zone 1). Porosity was significantly greater in PTH1 and PTH5 than in sham or OVX near the periosteal surface (Zone III) as well, but the increases were much smaller. Zone I is the inner (endocortical) one-third of the cortical diameter, Zone II is the intermediate one-third, and Zone III is the outer (periosteal) one-third of the diameter. S =  $p < .05$  vs sham; O =  $p < .05$  vs OVX. Data are expressed as Mean  $\pm$  SEM. (B) If porosity were uniformly distributed throughout the cross-section, the reduction in strength of the humerus would have been greater than when the porosity is primarily distributed close to the endocortical surface, as it was in PTH-treated monkeys. For PTH1 and PTH5, a uniform distribution of porosity would have caused a significant decrease in strength of the humerus ( $* p < .05$ ). Because there was a gradient of porosity that decreased toward the periosteal surface, no significant reductions in strength were predicted. Data were expressed as Mean  $\pm$  SEM.

In a recently published paper by Burr et al. (J. Bone Miner. Res. 16(1), 2001) the issue of cortical porosity, its distribution and its effect on strength was addressed further. Data from the current monkey study on architecture and strength of the humerus midshaft were used to support the hypothesis that the dose-dependent increase in cortical porosity upon intermittent administration of PTH(1-34) does not reduce the strength or stiffness of cortical bone. According to the data, PTH (1 and 5 µg/kg/day) dose-dependently increased cortical bone turnover and intracortical porosity. This would be expected to result in a decrease in cortical BMD. The increase in porosity occurred mainly in the endocortical zone. This increase in porosity is theoretically expected to result in a decrease in bone strength (based on an estimation of Young's modulus in each zone). However, the decrease would not be expected to be as large as when porosity would have been uniformly distributed throughout the cortical zones.

The data also showed that PTH caused addition of new bone and increased cortical area and thickness and increased bone formation at the endocortical surface, which was statistically significant

at the high dose (see Table E4). This would offset the adverse effect of increased porosity on strength. Actual strength measurements confirmed the predictions based on bone architecture (see Table B4, above). The effect of PTH withdrawal for 6 months was that at the low dose the increased porosity was reversed to near OVX levels, while at the high dose it was partially maintained (Table E4). The absence of reversal in the endocortical zone could indicate a temporary uncoupling of resorption and formation. However, strength measurements did not suggest adverse effects after PTH withdrawal at either dose.

It should be noted that in the published study only at the high dose of PTH a significantly increased thickness of cortical bone was observed. Treatment with the low dose, which is paralleled by a plasma AUC of PTH(1-34) similar to the expected AUC in humans treated with 20 ug/day, did not significantly affect cortical thickness. This raises concern about the generalization of the humerus findings. It is conceivable that at the intended human dose, an increase in endocortical porosity and an associated decrease in cortical bone BMD leads to decreased cortical bone strength (when the increased porosity is not balanced by a sufficient increase in bone thickness). Nevertheless, in the current monkey study the humeral strength was not significantly affected/decreased at the low dose (Table B4), possibly because there was a slight non-significant increase in thickness that balanced the increased porosity. However, since the humerus was the only cortical bone site assessed for strength, the theory that PTH will not pose an increased cortical fracture risk may not be applicable to all cortical bone sites.

In fact, histomorphometry data for the midshaft radius from this study showed that PTH causes a slight decrease in BV/TV while bone area (ie thickness) was not significantly affected (Table F7). Bone densitometry also showed a decrease in BMD). However, according to this technique X-area was dose-dependently increased. A decrease in BV/TV also occurred in the cortical part of the distal radius (Table G3) while BMD of this site was slightly decreased by PTH1 in the middle zones. Therefore, it is unclear whether this bone site may be subject to deleterious effects of PTH on bone strength. Histomorphometry data on the femoral midshaft (Table F10) suggested that BV/TV and B.Ar (thickness) were not affected by PTH.

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## Mid-Radius, Mid-Femur, Vertebra, and Femoral Neck (18-month data)

Table F1 Definition of Histomorphometric Variables for Cortical Bone Measurements of the Midshaft Femur and Radius<sup>a</sup>

Variable	Units	Description
T.Ar	mm <sup>2</sup>	Tissue area, the total area within the periosteal surface
Ma.Ar	mm <sup>2</sup>	Marrow area, the area of the medullary cavity
Md.Ar	mm <sup>2</sup>	Mineralized area, the area of mineralized bone, excluding porosities
Vd.Ar	mm <sup>2</sup>	Void area, the area of porosities in the cortex
Ps.Pm	mm	Periosteal bone perimeter
On.Pm	mm	Osteonal bone perimeter
Ec.Pm	mm	Endocortical bone perimeter
Po	%	Porosity, the percentage of mineralized area occupied by porosities
Md V/TV	%	Bone volume, mineralized area as a percentage of tissue area
Ps.MS/Md S	%	Periosteal mineralizing surface
Ps.MAR	µm/day	Periosteal mineral apposition rate
Ps.BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Periosteal bone formation rate, surface referent
Ps.BFR/Md.V	%/year	Periosteal bone formation rate, bone volume referent
On.MS/Md.S	%	Osteonal mineralizing surface
On.MAR	µm/day	Osteonal mineral apposition rate
On.BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Osteonal bone formation rate, surface referent
On.BFR/Md.V	%/year	Osteonal bone formation rate, bone volume referent
Ec.MS/Md.S	%	Endocortical mineralizing surface
Ec.MAR	µm/day	Endocortical mineral apposition rate
Ec.BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Endocortical bone formation rate, surface referent
Ec.BFR/Md.V	%/year	Endocortical bone formation rate, bone volume referent
Tt.BFR/Md.V	%/year	Sum of periosteal, osteonal, and endocortical BFR/BV

<sup>a</sup> Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J Bone Miner Res* 2:595-610.

Table F2 Definition of Histomorphometric Variables for Structural and Label Measurements of Vertebra and Femur Neck<sup>a</sup>

Variable	Units	Description
Md V/TV	%	Bone volume, the mineralized area as a percentage of tissue area
Md.S/Md.V	mm <sup>2</sup> /mm <sup>3</sup>	Mineralized tissue surface:volume ratio
Tb.Th	µm	Trabecular thickness
Tb.N	mm <sup>-1</sup>	Trabecular number
Tb.Sp	µm	Trabecular separation
MS/Md.S	%	Mineralizing surface
MAR	µm/day	Mineral apposition rate
BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Bone formation rate, surface referent
BFR/Md.V	%/year	Bone formation rate, bone volume referent
BFR/TV	%/year	Bone formation rate, tissue volume referent
Ps.MS/Md.S	%	Periosteal mineralizing surface
Ps.MAR	µm/day	Periosteal mineral apposition rate
Ps.BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Periosteal bone formation rate, surface referent

<sup>a</sup> Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J Bone Miner Res* 2:595-610.

Table F3 Definition of Histomorphometric Variables for Histological Measurements of the Vertebra<sup>a</sup>

Variable	Units	Description
BV/TV	%	Bone volume as a percentage of tissue volume
BS/BV	mm <sup>2</sup> /mm <sup>3</sup>	Bone surface:volume ratio
Tb.Th	µm	Trabecular thickness
Tb.N	mm <sup>-1</sup>	Trabecular number
Tb.Sp	µm	Trabecular separation
It.Th	µm	Interstitial thickness
OV/BV	%	Osteoid volume as a percentage of bone volume
OV/TV	%	Osteoid volume as a percentage of tissue volume
O.Th	µm	Osteoid thickness
W.Th	µm	Wall thickness, the thickness of completed bone packets
OS/BS	%	Osteoid surface as a percentage of bone surface
ES/BS	%	Eroded surface as a percentage of bone surface
Oc.S/BS	%	Osteoclastic surface as a percentage of bone surface
Ac.F	cycles/year	Activation frequency
Aj.AR	µm/day	Adjusted apposition rate
FP	days	Formation period
Rs.P	days	Resorption (osteoclastic) period
Rv.P	days	Reversal period
Mlt	days	Mineralization lag time
Omt	days	Osteoid maturation time

<sup>a</sup> Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J Bone Miner Res* 2:595-610.

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Mid-Radius (Table F7):

Measurements of static and dynamic parameters in cortical bone from the midshaft radius:

Effect of OVX (relative to sham):

- Significant increases in Po, On.MS/Md.S, On.BFR/Md.S, On.BFR/Md.V
- N.s. increase in Vd.Ar, On.Pm, Ps.MS/Md.S, Ps.BFR/Md.S, Ps.BFR/Md.V, and Ec.BFR-parameters
- No effect on T.Ar, Ma.Ar, Md.Ar (bone area), Md.V/TV

Effect of LY treatment (relative to OVX):

- Significant increases in T.Ar, Ma.Ar, Vd.Ar, On.Pm, Ec.Pm, Po, On.BFR/Md.V, Ec.MS/Md.S, Ec.BFR/Md.S and Ec.BFR/Md.V
- Significant decrease in Md.V/TV (=BV/TV) !
- N.s. slight increases in Ps.BFR/Md.S and Ps.BFR/Md.V
- No effect on Md.Ar

Effect of LY treatment withdrawal (relative to OVX):

- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e. VdAr, On.Pm, Po, Ps.Ms/Md.S, Ps.BFR/Md.S, Ps.BFR/Md.V, On.BFR/Md.S, On.BFR/Md.V, and Ec.BFR parameters)
- No further effect on or reversal of decreased Md.V/TV (BV/TV). No effect on Md.Ar

Conclusion: Ovariectomy caused increased cortical bone turnover and porosity but had no effect on bone area. PTH caused increased endocortical and osteonal bone formation and increased cortical porosity. PTH decreased fractional bone volume (Md.V/TV), but did not affect total bone area. PTH withdrawal decreased periosteal bone formation to below OVX levels! A similar finding was obtained in the humerus midshaft. However, withdrawal did not reverse the decrease in fractional bone volume (BV/TV) or bone area.

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Note: 3 out of 21 mid-radius specimens from the PTH5 group had intramedullary bone formation (ring of mineralized bone under endocortical surface present in the medullary area)

Table F7 Static and Dynamic Histomorphometry of the Midshaft Radius (n = 121, Mean ± SEM of Raw Data) and Bonferroni-Adjusted Group Comparisons (128-Monkey Population)

	SEM n = 19 <sup>a</sup>	CVX n = 19 <sup>b</sup>	PTH1 n = 19 <sup>c</sup>	PTH1-W n = 19 <sup>d</sup>	PTH5 n = 21 <sup>e</sup>	PTH5-W n = 21 <sup>f</sup>	Tissue formation	Over- all p	Bonferroni-Adjusted p CVX (%)				
									Sham	PTH1	PTH5	PTH5-W	
T Ar	20.4 ± 0.4	19.2 ± 0.5	21.2 ± 0.6	20.8 ± 0.5	21.0 ± 0.4	21.0 ± 0.6		.06	ns	<.05	ns	<.05	<.1
MS Ar	4.70 ± 0.30	3.75 ± 0.26	4.94 ± 0.29	5.06 ± 0.31	4.51 ± 0.32	5.13 ± 0.33		.02	ns	<.05	<.05	ns	<.01
MS Ar	15.3 ± 0.5	14.9 ± 0.3	15.3 ± 0.4	15.2 ± 0.3	15.3 ± 0.3	14.9 ± 0.5		.93	ns	ns	ns	ns	ns
MS Ar	0.35 ± 0.05	0.57 ± 0.05	0.97 ± 0.15	0.60 ± 0.05	1.21 ± 0.13	0.91 ± 0.15	*	.00	ns	ns	ns	<.05	ns
Ps Pm	17.2 ± 0.2	16.9 ± 0.3	17.4 ± 0.3	17.3 ± 0.3	17.4 ± 0.2	17.4 ± 0.3		.06	ns	ns	ns	ns	ns
Os Pm	26.6 ± 1.6	33.3 ± 2.1	42.4 ± 3.4	33.1 ± 2.2	32.5 ± 3.1	30.7 ± 3.1		.00	ns	<.1	ns	<.001	ns
Ec Pm	7.79 ± 0.29	6.79 ± 0.32	8.19 ± 0.33	8.10 ± 0.27	7.82 ± 0.36	8.66 ± 0.42		.01	ns	<.05	<.05	ns	<.001
PO	2.95 ± 0.43	3.67 ± 0.35	5.00 ± 0.82	3.05 ± 0.33	7.21 ± 0.71	5.61 ± 1.13	log	.00	<.05	ns	ns	<.01	ns
MS V TV	75.2 ± 1.6	77.8 ± 1.1	72.3 ± 1.4	73.5 ± 1.2	73.1 ± 1.3	71.5 ± 1.5		.02	ns	<.05	ns	<.1	<.01
Ps MS Mid S	4.9 ± 1.3	12.0 ± 3.4	13.4 ± 3.1	7.4 ± 1.3	13.5 ± 2.8	5.3 ± 1.1	square root	.01	<.1	ns	ns	ns	ns
Ps MAR	0.71 ± 0.12	0.51 ± 0.05	0.57 ± 0.06	0.70 ± 0.12	0.61 ± 0.05	0.61 ± 0.07	*	.82	ns	ns	ns	ns	ns
Ps BFR Mid S	12.8 ± 3.4	28.0 ± 9.6	36.9 ± 8.0	21.0 ± 4.2	33.2 ± 7.2	13.4 ± 3.9	square root	.05	ns	ns	ns	ns	ns
Ps BFR Mid V	1.56 ± 0.42	3.21 ± 1.05	3.51 ± 0.90	2.00 ± 0.49	3.89 ± 0.87	1.55 ± 0.45	square root	.07	ns	ns	ns	ns	ns
Os MS Mid S	6.3 ± 1.1	26.3 ± 2.6	30.1 ± 1.7	24.0 ± 3.0	32.2 ± 1.9	20.3 ± 2.4	*	.00	<.05	ns	ns	ns	ns
Os MAR	0.95 ± 0.06	1.14 ± 0.04	0.99 ± 0.03	1.10 ± 0.04	1.01 ± 0.02	1.04 ± 0.03	*	.01	<.1	ns	ns	ns	ns
Os BFR Mid S	24 ± 5	104 ± 10	107 ± 5	97 ± 12	110 ± 7	79 ± 11	*	.00	<.05	ns	ns	ns	ns
Os BFR Mid V	4.7 ± 1.0	24.4 ± 3.3	29.7 ± 2.4	22.3 ± 3.3	41.0 ± 3.4	19.9 ± 2.7	square root	.00	<.001	ns	ns	<.01	ns
Ec MS Mid S	2.3 ± 1.7	12.1 ± 4.2	19.7 ± 6.7	13.2 ± 4.3	34.7 ± 5.7	15.0 ± 4.3	*	.00	ns	ns	ns	<.05	ns
Ec MAR	0.47 ± 0.17	0.75 ± 0.09	0.65 ± 0.09	0.73 ± 0.06	0.79 ± 0.07	0.66 ± 0.06	*	.54	ns	ns	ns	ns	ns
Ec BFR Mid S	6.7 ± 5.9	37.2 ± 13.2	60.5 ± 23.6	39.2 ± 13.1	111.9 ± 22.6	48.0 ± 16.6	*	.00	ns	ns	ns	<.1	ns
Ec BFR Mid V	0.24 ± 0.10	1.01 ± 0.67	3.55 ± 1.37	2.83 ± 0.64	6.23 ± 1.36	3.21 ± 1.12	*	.00	ns	ns	ns	<.05	ns
Ti BFR Mid V	6.6 ± 1.4	29.5 ± 4.1	36.8 ± 3.1	26.8 ± 4.0	51.1 ± 4.2	24.7 ± 3.2	square root	.00	<.001	ns	ns	<.001	ns

n = p - 1

<sup>a</sup> Exceptions: Ps MAR (n = 16), Os MAR (n = 16) and Ec MAR (n = 4)

<sup>b</sup> Exceptions: Ps MAR (n = 18) and Ec MAR (n = 11)

<sup>c</sup> Exceptions: Ec MAR (n = 13)

<sup>d</sup> Exceptions: Ec MAR (n = 10)

<sup>e</sup> Exceptions: Ps MAR (n = 21) and Ec MAR (n = 21)

<sup>f</sup> Exceptions: Ps MAR (n = 18) and Ec MAR (n = 14)

\* Data resistant to transformation, analyzed using Kruskal-Wallis test

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Midshaft Femur (Table F10):

Measurements of static and dynamic parameters in midshaft femur

Effect of OVX (relative to sham):

- Significant increases in On.Pm, Po, Ps.MS/BS, Ps.BFR, On.MS/BS, On.MAR, On.BFR/BS, On.BFR/BV, and Ec.BFR parameters
- N.s. increase in Vd.Ar, Ps.MAR
- No effect on T.Ar, Ma.Ar, B.Ar, BV/TV

Effect of LY treatment (relative to OVX):

- Significant increases in Vd.Ar, On.Pm, Po, On.MS/BS, On.BFR/BS, On.BFR/BV, and Ec.BFR parameters
- N.s. increase in B.Ar
- N.s. increases in Ps.MS/BS, Ps.BFR/BS and Ps.BFR/BV at high dose only
- No effect on T.Ar, Ma.Ar, BV/TV !

Effect of LY treatment withdrawal (relative to OVX):

- Some parameters were reversed significantly below OVX levels (Ps.MS/BS, Ps.BFR/BS, Ps.BFR/BV, On.MAR, On.BFR/BS)
- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., Vd.Ar, On.Pm, Po, On.BFR/BV, Ec. BFR parameters)
- No effect on T.Ar, Ma.Ar, B.Ar, BV/TV

Conclusion: Ovariectomy caused increased cortical bone turnover and porosity but did not affect bone area. PTH caused increased endocortical, periosteal and osteonal bone formation and increased cortical porosity. However, PTH did not affect fractional bone volume (BV/TV) and slightly increased total bone area. PTH withdrawal decreased bone formation parameters to below OVX (near-sham) levels. PTH withdrawal did not affect specific bone volume (BV/TV) or total bone area. Similar findings were obtained in the humerus and midshaft radius.

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**Table F10 Static and Dynamic Histomorphometry of the Midshaft Femur (n = 121, Mean ± SEM of Raw Data) and Bonferroni-Adjusted Group Comparisons (121-Monkey Population)**

	Min n = 21 <sup>a</sup>	OV n = 21 <sup>b</sup>	PTH n = 11	PTH-W n = 21 <sup>c</sup>	PTH n = 21	PTH-S-W n = 21 <sup>d</sup>	Trans-formation	Over-		Bonferroni-Adjusted p.			
								all	p	Sham	PTH	PTH-W	PTH <sup>e</sup>
T.Ar	50.5 ± 1.0	51.2 ± 1.6	51.7 ± 0.9	53.4 ± 1.4	53.2 ± 1.1	52.5 ± 1.4		.51	ns	ns	ns	ns	ns
Ma.Ar	17.3 ± 0.8	18.9 ± 1.0	17.5 ± 0.7	19.7 ± 1.0	17.1 ± 1.0	17.0 ± 1.2	log	.14	ns	ns	ns	ns	ns
B.Ar	32.9 ± 0.7	31.5 ± 1.0	32.5 ± 0.8	33.0 ± 0.7	33.7 ± 0.6	33.8 ± 0.8		.29	ns	ns	ns	ns	ns
Vd.Ar	0.36 ± 0.03	0.76 ± 0.10	1.69 ± 0.37	0.73 ± 0.14	2.48 ± 0.43	1.73 ± 0.41		.00	<.1	ns	ns	<.05	ns
Ps.Pm	25.2 ± 0.3	25.5 ± 0.4	25.7 ± 0.2	26.1 ± 0.4	26.1 ± 0.3	25.9 ± 0.4		.30	ns	ns	ns	ns	ns
Os.Pm	36.1 ± 2.7	51.9 ± 3.7	70.2 ± 5.8	56.0 ± 5.7	93.9 ± 8.4	67.9 ± 5.8	log	.00	<.05	<.1	ns	<.001	ns
Ln.Pm	14.7 ± 0.8	15.7 ± 0.4	14.8 ± 0.4	16.2 ± 0.6	14.8 ± 0.5	14.6 ± 0.5		.09	ns	ns	ns	ns	ns
Pu	1.09 ± 0.10	2.42 ± 0.33	4.78 ± 0.96	2.87 ± 0.36	6.55 ± 1.04	4.77 ± 1.06		.00	<.05	ns	ns	<.05	ns
BV.TV	65.2 ± 1.1	61.0 ± 1.1	62.9 ± 1.2	62.1 ± 1.1	63.6 ± 1.3	64.8 ± 1.5		.23	ns	ns	ns	ns	ns
Ps.MS.BS	2.2 ± 0.6	12.5 ± 3.1	12.1 ± 2.0	4.0 ± 1.2	22.8 ± 4.0	1.8 ± 0.7		.00	<.05	ns	ns	ns	<.05
Ps.MAR	0.54 ± 0.07	0.76 ± 0.07	0.62 ± 0.07	0.50 ± 0.09	0.80 ± 0.05	0.69 ± 0.07		.01	ns	ns	ns	ns	ns
Ps.BFR.BS	5.6 ± 2.3	41.7 ± 12.8	31.7 ± 6.0	11.7 ± 5.1	79.6 ± 15.0	3.3 ± 1.1		.00	<.05	ns	ns	ns	<.05
Ps.BFR.BV	0.47 ± 0.20	3.57 ± 1.12	2.54 ± 0.47	0.96 ± 0.43	9.87 ± 1.18	0.25 ± 0.08		.00	<.05	ns	ns	ns	<.05
Os.MS.BS	5.3 ± 1.5	17.0 ± 2.4	19.0 ± 2.1	9.4 ± 1.5	27.4 ± 2.1	11.5 ± 1.6	square root	.00	<.001	ns	<.1	<.01	ns
Os.MAR	0.91 ± 0.12	1.28 ± 0.04	1.88 ± 0.03	1.12 ± 0.06	1.14 ± 0.03	1.84 ± 0.03		.00	<.05	<.05	ns	ns	<.05
Os.BFR.BS	21.6 ± 6.4	77.9 ± 10.7	75.9 ± 8.9	39.8 ± 6.3	114.0 ± 9.4	43.8 ± 5.9		.00	<.001	ns	<.01	<.01	<.05
Os.BFR.BV	2.8 ± 1.0	14.6 ± 2.8	18.2 ± 3.1	7.0 ± 1.3	33.7 ± 4.7	9.2 ± 1.6	square root	.00	<.001	ns	ns	<.001	ns
Ec.MS.BS	5.5 ± 2.7	12.9 ± 3.7	29.7 ± 5.0	6.8 ± 1.3	38.0 ± 5.8	5.0 ± 2.0		.00	ns	<.1	ns	<.05	ns
Ec.MAR	0.44 ± 0.06	0.58 ± 0.08	0.68 ± 0.06	0.59 ± 0.10	0.78 ± 0.06	0.51 ± 0.09		.01	ns	ns	ns	ns	ns
Ec.BFR.BS	13.2 ± 7.6	38.0 ± 14.3	85.0 ± 15.4	16.2 ± 4.1	124.2 ± 25.8	14.8 ± 5.9		.00	ns	<.1	ns	<.05	ns
Ec.BFR.BV	0.29 ± 0.22	2.00 ± 0.77	3.73 ± 0.77	0.75 ± 0.38	5.33 ± 1.06	0.66 ± 0.27		.00	ns	ns	ns	<.05	ns
Tt.BFR.BV	3.9 ± 1.2	20.2 ± 3.4	24.5 ± 3.5	8.7 ± 1.5	44.9 ± 5.9	10.2 ± 1.7		.00	<.05	ns	ns	<.1	ns

ns = p>.1

<sup>a</sup> Exceptions: Ps.MAR (n = 11), Os.MAR (n = 17) and Ec.MAR (n = 14)

<sup>b</sup> Exceptions: Ps.MAR (n = 17), Os.MAR (n = 19) and Ec.MAR (n = 17)

<sup>c</sup> Exceptions: Ps.MAR (n = 19) and Ec.MAR (n = 17)

<sup>d</sup> Exceptions: Ps.MAR (n = 15), Os.MAR (n = 19) and Ec.MAR (n = 12)

Data rescaled to transformation, analyzed using Kruskal-Wallis test

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LV2

(A) *Measurements of static and dynamic parameters in both cortical and cancellous vertebral bone (Table F12):*

Cortical bone:

Effect of OVX (relative to sham):

- Significant increases in BFR/Md.S, BFR/Md.V, BFR/TV
- N.s. increase in Md.S/Md.V, MS/Md.S, MAR, Ps.MS/Md.S, Ps.BFR/Md.S
- No effect on Md.V/TV (=BV/TV)

Effect of LY treatment (relative to OVX):

- Significant increases in Md.V/TV, BFR/TV (increase in BV/TV also observed with DEXA)
- N.s. increases in Md.S/Md.V, MS/Md.S, BFR/Md.S, BFR/Md.V, Ps.MS/Md.S, Ps.BFR/Md.S
- No effect on MAR

Effect of LY treatment withdrawal (relative to OVX):

- Parameters were reversed towards OVX levels (static) or below-OVX levels (dynamic), but were not significantly different from OVX (i.e., Md.V/TV, Md.S/Md.V, MS/Md.S, BFR's, Ps.MS/Md.S, Ps.BFR/Md.S)

Conclusion: OVX increased cortical and periosteal bone formation, but not cortical bone volume density. PTH increased both cortical and periosteal bone formation and increased cortical bone volume density. PTH withdrawal caused reversal of cortical and periosteal bone formation to below-OVX levels, and reversal of bone volume density to above-OVX levels.

Cancellous bone:

Effect of OVX (relative to sham):

- No significant effects

Effect of LY treatment (relative to OVX):

- Significant increases in Md.V/TV, Tb.N., MS/Md.S, BFR's
- Significant decrease in Tb.Sp.
- N.s. increases in Tb.Th

Effect of LY treatment withdrawal (relative to OVX):

- Static parameters were reversed towards OVX levels (i.e., Md.V/TV, Tb.Th, Tb.N., Tb.Sp.). Dynamic parameters (MS/Md.S, BFR's) were reversed but did not reach levels below OVX as was seen in cortical bone

Conclusion: Ovariectomy had no effect on cancellous bone turnover, volume density or trabecular parameters. PTH caused increased trabecular density, cancellous bone density and cancellous bone formation parameters. PTH withdrawal caused partial reversal of these parameters. There were no specific data on the effect of PTH on bone resorption.

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*(B) Measurements of histology/label-derived data in LV2 cancellous bone (Table F14):*

Effect of OVX (relative to sham):

- N.s. increase in O.Th, Oc.S/BS, FP, Rs.P, Mit
- No effects on BV/TV or trabecular parameters

Effect of LY treatment (relative to OVX):

- Significant increases in BV/TV, Tb.N., Ac.F
- Significant decreases in Tb.Sp, O.Th
- N.s. increase in OV/TV, OS/BS
- N.s. decrease in Oc.S/BS, but no effect on ES/BS
- N.s. decreases in FP, Rs.P

Effect of LY treatment withdrawal (relative to OVX):

- Some parameters were reversed towards OVX levels, but were not significantly different from OVX (i.e., BV/TV, Tb.N., Tb.Sp, OV/TV, O.Th)

Conclusion: Ovariectomy caused an increase in relative osteoclast surface, but did not affect cancellous bone turnover, bone volume or trabecular density. Ovariectomy lengthened the remodeling period. PTH increased cancellous bone formation, bone volume and trabecular density. PTH decreased osteoid thickness. The effect on bone resorption was unclear. PTH withdrawal caused partial reversal of the increased bone volume and trabecular density, and the decreased osteoid thickness. The nature of the effect of PTH on osteoid thickness is unclear.

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**Table F12 Static and Dynamic Histomorphometry of LV2 (n = 118, Mean ± SEM of Raw Data) and Bonferroni-Adjusted Group Comparisons (121-Monkey Population)**

Tissue	Group	Sum n = 9 <sup>a</sup>	CVX n = 10 <sup>b</sup>	PDU n = 9 <sup>c</sup>	PTH-W n = 10 <sup>d</sup>	PTIS n = 10 <sup>e</sup>	PTB-W n = 9 <sup>f</sup>	Trans-formation	Over-all p	Bonferroni-Adjusted p, OVA vs.				
										Sham	PTH	PTH-W	PTH-W	
MDV-TN	Cortical	66.2 ± 1.9	62.8 ± 2.4	69.2 ± 2.0	65.5 ± 1.7	72.1 ± 1.5	67.6 ± 1.8		.01	ns	<.05	ns	<.01	ns
	Cancellous	26 ± 2	23 ± 1	33 ± 1	36 ± 1	35 ± 1	27 ± 1	log	.00	ns	<.001	<.001	<.001	<.05
Mdn-MdV	Cortical	6.2 ± 0.3	7.5 ± 0.5	8.6 ± 0.7	7.2 ± 0.4	8.2 ± 0.4	7.2 ± 0.5		.01	ns	ns	ns	ns	ns
	Cancellous	22 ± 1	22 ± 1	21 ± 1	20 ± 1	21 ± 1	22 ± 1		.35	ns	ns	ns	ns	ns
Tb-Th	Cancellous	95 ± 3	93 ± 3	97 ± 3	103 ± 5	100 ± 4	94 ± 3	reciprocal	.35	ns	ns	ns	ns	ns
Tb-N	Cancellous	2.7 ± 0.1	2.8 ± 0.1	3.4 ± 0.1	2.9 ± 0.1	3.6 ± 0.2	2.9 ± 0.1	log	.00	ns	<.001	<.05	<.001	<.05
Tb-Sp	Cancellous	294 ± 16	313 ± 15	292 ± 8	250 ± 14	195 ± 13	253 ± 10		.00	ns	<.01	<.01	<.001	<.01
MS-MdS	Cortical	16.2 ± 1.9	22.7 ± 2.0	26.4 ± 2.3	19.2 ± 1.6	30.0 ± 2.3	18.4 ± 2.3		.00	ns	ns	ns	<.1	ns
	Cancellous	15 ± 2	15 ± 2	22 ± 3	16 ± 3	29 ± 2	17 ± 2		.00	ns	ns	ns	ns	<.001
MAR	Cortical	0.64 ± 0.03	0.76 ± 0.04	0.81 ± 0.06	0.75 ± 0.04	0.74 ± 0.06	0.77 ± 0.04		.05	ns	ns	ns	ns	ns
	Cancellous	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0		.02	ns	ns	ns	ns	ns
BFR-MdS	Cortical	39.1 ± 5.0	64.8 ± 7.0	77.0 ± 10.0	54.9 ± 5.9	78.3 ± 7.4	49.4 ± 5.8		.06	<.05	ns	ns	ns	ns
	Cancellous	41 ± 6	39 ± 5	52 ± 7	45 ± 7	64 ± 6	42 ± 4	log	.07	ns	ns	ns	ns	<.05
BFR-MdV	Cortical	25 ± 4	50 ± 6	72 ± 17	40 ± 5	65 ± 7	34 ± 4	square root	.00	<.05	ns	ns	ns	ns
	Cancellous	89 ± 19	84 ± 10	112 ± 16	84 ± 12	135 ± 14	88 ± 10		.05	ns	ns	ns	ns	<.05
BFR-TV	Cortical	18.0 ± 2.2	29.6 ± 3.6	46.6 ± 9.1	25.8 ± 3.1	47.0 ± 5.5	22.8 ± 2.6	square root	.00	<.05	<.1	ns	ns	<.05
	Cancellous	23 ± 4	20 ± 2	36 ± 5	26 ± 4	47 ± 5	24 ± 2	square root	.00	ns	<.05	ns	ns	<.001
P-MN-MdS	Cortical	3.0 ± 1.5	9.3 ± 2.2	8.0 ± 2.5	4.6 ± 1.6	13.1 ± 2.3	4.2 ± 0.8		.06	<.1	ns	ns	ns	ns
P-MAR	Cortical	0.38 ± 0.06	0.49 ± 0.06	0.54 ± 0.06	0.42 ± 0.05	0.39 ± 0.05	0.40 ± 0.04		.53	ns	ns	ns	ns	ns
P-BFR-MdS	Cortical	4.0 ± 1.3	16.5 ± 4.6	17.0 ± 5.5	7.8 ± 3.1	21.0 ± 5.0	6.6 ± 1.5		.06	<.1	ns	ns	ns	ns

ns = p > .1

<sup>a</sup> Exception: Ps-MAR (n = 11)

<sup>c</sup> Exception: Ps-MAR (n = 19)

<sup>b</sup> Exception: Ps-MAR (n = 17)

<sup>e</sup> Exception: Ps-MAR (n = 16)

<sup>d</sup> Exception: Ps-MAR (n = 15)

<sup>f</sup> Data resistant to transformation, analyzed using Kruskal-Wallis test

<sup>e</sup> Exception: Ps-MAR (n = 13)

**Table F14 LV2 Cancellous Histological and Combined Histological/Label Derived Data (n = 118, Mean ± SEM of Raw Data) and Bonferroni-Adjusted Group Comparisons (121-Monkey Population)**

Tissue	Group	Sum n = 19	CVX n = 19	PDU n = 9	PTH-W n = 10	PTIS n = 10	PTB-W n = 10	Trans-formation	Over-all p	Bonferroni-Adjusted p, OVA vs.				
										Sham	PTH	PTH-W	PTH-W	
OV-TN		25.4 ± 1.4	26.1 ± 1.0	36.0 ± 1.4	30.2 ± 1.1	36.3 ± 1.5	29.6 ± 1.1		.06	ns	<.001	ns	<.001	ns
BN-BV		19.7 ± 0.6	18.6 ± 0.6	18.0 ± 0.6	18.3 ± 0.6	18.3 ± 0.6	19.5 ± 0.7		.24	ns	ns	ns	ns	ns
Tb-Th		103 ± 3	110 ± 3	114 ± 4	112 ± 4	111 ± 3	105 ± 4		.24	ns	ns	ns	ns	ns
Tb-N		2.45 ± 0.10	2.41 ± 0.09	3.20 ± 0.11	2.72 ± 0.10	3.30 ± 0.15	2.85 ± 0.12		.06	ns	<.001	ns	<.001	<.05
Tb-Sp		319 ± 20	320 ± 18	285 ± 9	264 ± 12	264 ± 14	257 ± 13	log	.00	ns	<.001	<.1	<.001	<.05
h-Th		51.4 ± 2.7	55.0 ± 3.1	59.6 ± 4.1	59.1 ± 3.9	56.8 ± 3.5	54.2 ± 3.1	log	.54	ns	ns	ns	ns	ns
OV-BV		2.07 ± 0.45	2.24 ± 0.25	1.91 ± 0.31	1.30 ± 0.17	2.30 ± 0.34	1.84 ± 0.29	log	.22	ns	ns	<.05	ns	ns
OV-TV		0.50 ± 0.16	0.59 ± 0.07	0.70 ± 0.12	0.43 ± 0.06	0.85 ± 0.14	0.54 ± 0.09	square root	.09	ns	ns	ns	ns	ns
C-Tb		6.44 ± 0.56	7.10 ± 0.27	6.37 ± 0.25	6.54 ± 0.27	5.57 ± 0.15	6.17 ± 0.27		.01	ns	ns	ns	<.05	ns
W-Tb		25.9 ± 0.5	27.3 ± 0.8	27.0 ± 0.8	26.5 ± 0.7	27.3 ± 0.5	25.4 ± 0.6		.13	ns	ns	ns	ns	ns
US-BS		15.4 ± 2.2	16.7 ± 1.8	15.5 ± 2.0	11.6 ± 1.6	21.9 ± 3.1	15.0 ± 2.2	square root	.05	ns	ns	ns	ns	ns
LS-BS		53.2 ± 3.5	43.1 ± 2.8	44.2 ± 2.1	47.7 ± 2.5	47.9 ± 2.7	49.5 ± 2.2		.11	ns	ns	ns	ns	ns
OKS-BS		1.30 ± 0.19	1.74 ± 0.23	1.31 ± 0.15	1.55 ± 0.25	1.43 ± 0.14	1.70 ± 0.27	square root	.61	ns	ns	ns	ns	ns
Ac-F		1.98 ± 0.38	1.79 ± 0.21	2.43 ± 0.29	2.17 ± 0.34	3.00 ± 0.27	2.09 ± 0.23	log	.04	ns	ns	ns	ns	<.05
Ap-MAR		0.82 ± 0.12	0.73 ± 0.08	1.17 ± 0.23	1.21 ± 0.17	1.00 ± 0.11	0.96 ± 0.13	log	.06	ns	ns	<.05	ns	ns
FP		33.6 ± 4.2	45.7 ± 11.6	27.0 ± 4.6	23.5 ± 3.1	26.3 ± 2.7	27.3 ± 3.6	log	.06	ns	ns	<.05	ns	ns
Ra-P		3.45 ± 0.75	5.83 ± 1.02	2.77 ± 0.61	9.51 ± 0.62	2.01 ± 0.26	4.28 ± 1.07		.09	ns	ns	ns	ns	<.1
Rv-P		157 ± 30	114 ± 17	96 ± 22	237 ± 142	72 ± 10	123 ± 23	log	.11	ns	ns	ns	ns	ns
Mh		11.3 ± 2.4	14.6 ± 3.2	8.2 ± 1.5	7.5 ± 1.1	7.0 ± 0.8	8.1 ± 0.9	log	.01	ns	<.05	<.05	<.05	ns
Low		10.5 ± 1.1	10.7 ± 0.4	9.9 ± 0.5	9.5 ± 0.6	9.5 ± 0.4	9.1 ± 0.4		.45	ns	ns	ns	ns	ns

<sup>a</sup> More than 25% of the groups nonnormally distributed and transformation was ineffective, analyzed using Kruskal-Wallis test

<sup>b</sup> Results of Levene's test on the variable indicate a lack of homogeneity of variances (p < .05) that was not corrected by transformation, analyzed using Kruskal-Wallis test

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### Qualitative evaluation of vertebrae

#### Bright field examination:

- In 4 of 121 animals (2 PTH1, 2 PTH5) there was focal or diffuse osteoidosis (thickened osteoid) (osteoid = organic unmineralized bone matrix) or osteomalacia (=impaired mineralization with excess osteoid accumulation) in the vertebral section.
- In 6 of 121 animals (scattered through groups) there were small ventral osteophytes (osseous outgrowths).
- Trabecular tunneling was seen in all groups (Table F6). The incidence of score (0) and score (+) tunneling was decreased, and of score (++) and score (+++) increased in PTH1 and PTH5 relative to OVX. Score (+++) tunneling occurred only in PTH treated. Withdrawal of PTH appeared to decrease the extent of tunneling.

#### Polarized light examination:

- No abnormalities of lamellar structure observed in sections with or without histological abnormalities (osteoidosis, osteophyte).

**Table F6                      Trabecular Tunneling Summary**

Trabecular Tunneling Score	Total Cases	Number of Cases in Group					
		Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W
-	31	11	8	1	7	1	3
+	35	3	7	3	10	0	12
++	36	5	5	10	4	7	5
+++	19	0	0	5	0	13	1

Conclusion: In a few animals, PTH caused osteoidosis/osteomalacia. PTH caused a marked but reversible increase in the incidence and severity of trabecular tunneling. There were no abnormalities in lamellar structure.

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Femoral neck

Measurements of static and dynamic parameters in femoral neck (Table F16):

*Note: No periosteal double label observed in any section*

Cortical bone:

Effect of OVX (relative to sham):

- Significant increase in Ps.MS/Md.S
- N.s. increase in MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV
- No effect on Md.V/TV, Md.S/Md.V

Effect of LY treatment (relative to OVX):

- Significant increase in Md.S/Md.V (BS/BV)
- N.s. increase in BFR/Md.V, BFR/TV, Ps.MS/Md.S
- No effect on Md.V/TV

Effect of LY treatment withdrawal (relative to OVX):

- Some parameters were reversed significantly below OVX levels (MS/Md.S, BFR/Md.S)
- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., BFR/Md.V, BFR/TV, Ps.MS/Md.S)

Conclusion: Ovariectomy and PTH both caused increases in cortical and periosteal bone formation but no changes in cortical bone volume density. Bone formation parameters were decreased by PTH withdrawal to OVX or below-OVX (i.e. sham) levels.

Cancellous bone:

Effect of OVX (relative to sham):

- Nearly significant increase in Tb.Sp
- N.s. decrease in Tb.Th, Tb.N.
- N.s. increase in MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV
- No effect on Md.V/TV, Md.S/Md.V

Effect of LY treatment (relative to OVX):

- Significant increase in Md.V/TV, Tb.N., MS/Md.S, BFR/TV
- Significant decrease in Tb.Sp
- N.s. increase in Tb.Th, BFR/Md.S, BFR/Md.V
- No effect on Md.S/Md.V

Effect of LY treatment withdrawal (relative to OVX):

- Parameters were reversed to OVX or below-OVX levels, but were not significantly different from OVX (i.e., Md.V/TV, Tb.Sp, MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV)

Conclusion: Ovariectomy increased cancellous bone formation and decreased trabecular density but had no net effect on cancellous bone volume density. PTH increased both cancellous bone formation and trabecular and bone volume density. PTH withdrawal reversed the increases in cancellous bone formation and bone volume density. There were no specific data on resorption.

**Table F16 Static and Dynamic Histomorphometry of the Femur Neck (n = 120, Mean ± SEM of Raw Data) and Bonferroni-Adjusted Group Comparisons (121-Monkey Population)**

Tissue	Group	n	Mean	SEM	OVX	PTH	PTH-W	PTH	PTH-W	Transformation	p	Bonferroni-Adjusted p						
												OVX vs Sham	PTH vs Sham	PTH-W vs Sham	PTH vs PTH-W			
Mid VTN	Cortical	14	91.7	± 1.1	90.7	± 0.8	92.0	± 1.3	94.3	± 0.5	91.3	± 1.3	ns	ns	ns	ns		
	Cancellous	19	43.8	± 1.7	39.8	± 1.7	43.5	± 1.8	47.8	± 2.2	46.7	± 1.9	ns	<.001	<.01	<.001	<.001	
Mid Med V	Cortical	19	3.7	± 0.2	3.9	± 0.2	3.1	± 0.2	4.1	± 0.2	4.5	± 0.2	ns	<.01	ns	ns	<.1	
	Cancellous	19	13.5	± 0.6	13.2	± 0.4	13.5	± 0.5	13.4	± 0.7	12.2	± 0.6	ns	ns	ns	ns	ns	
Th-Th	Cancellous	7	154	± 7	151	± 5	154	± 7	155	± 7	172	± 9	log	ns	ns	ns	ns	ns
	Cancellous	7	2.9	± 0.1	2.6	± 0.1	3.5	± 0.1	3.1	± 0.1	3.4	± 0.1	ns	<.001	<.02	<.001	<.001	
Th-Sp	Cancellous	11	281	± 11	288	± 14	134	± 7	175	± 12	132	± 5	log	ns	<.001	<.001	<.001	<.001
	Cancellous	11	17.3	± 2.2	25.1	± 4.8	21.0	± 2.2	12.8	± 2.0	26.2	± 2.5	square root	ns	ns	<.01	ns	<.1
MAR	Cortical	19	0.8	± 0.0	0.8	± 0.0	0.8	± 0.0	0.8	± 0.1	0.9	± 0.0	ns	ns	ns	ns	ns	
	Cancellous	19	0.7	± 0.0	0.8	± 0.0	0.7	± 0.0	0.8	± 0.0	0.7	± 0.0	ns	ns	ns	ns	ns	
BFR Mid S	Cortical	19	50.3	± 6.8	78.1	± 14.6	66.8	± 7.3	41.9	± 7.8	84.3	± 9.0	square root	ns	ns	<.05	ns	<.05
	Cancellous	19	37.2	± 6.2	58.6	± 6.7	58.6	± 8.4	44.3	± 7.2	82.8	± 9.5	ns	ns	ns	ns	<.05	ns
BFR Mid V	Cortical	19	18.6	± 2.4	31.3	± 5.4	33.9	± 4.3	18.2	± 3.6	38.7	± 5.1	square root	ns	ns	<.1	ns	ns
	Cancellous	19	49.4	± 7.8	65.9	± 6.5	77.2	± 11.9	58.2	± 5.3	107.8	± 14.8	square root	.01	ns	ns	ns	ns
BFR TV	Cortical	19	16.9	± 2.2	28.2	± 4.8	30.8	± 3.8	16.5	± 3.2	34.3	± 4.8	square root	ns	ns	<.1	ns	ns
	Cancellous	19	20.7	± 3.2	25.1	± 3.6	42.1	± 7.3	26.8	± 4.1	59.8	± 7.7	square root	ns	ns	ns	<.01	ns
BAMS Mid S	Cortical	19	6.9	± 1.8	28.2	± 7.4	22.2	± 2.7	17.1	± 2.7	24.7	± 2.7	ns	<.01	ns	ns	ns	ns

ns = p > .1

\*Data requires to transformation, analyzed using Kruskal-Wallis test

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## Distal Radius

**Table G1** Definition of Histomorphometric Variables for Structural and Label Measurements of Distal Radius<sup>a</sup>

Variable	Units	Description
Md.V/TV	%	Bone volume, the mineralized area as a percentage of tissue area
Md.S/Md.V	mm <sup>3</sup> /mm <sup>3</sup>	Mineralized tissue surface/volume ratio
Tb.Th	µm	Trabecular thickness
Tb.N	mm <sup>-1</sup>	Trabecular number
Tb.Sp	µm	Trabecular separation
MS/Md.S	%	Mineralizing surface
MAR	µm/day	Mineral apposition rate
BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Bone formation rate, surface referent
BFR/Md.V	%/year	Bone formation rate, bone volume referent
BFR/TV	%/year	Bone formation rate, tissue volume referent
Ps.MS/Md.S	%	Periosteal mineralizing surface
Ps.MAR	µm/day	Periosteal mineral apposition rate
Ps.BFR/Md.S	mm <sup>3</sup> /mm <sup>2</sup> /day	Periosteal bone formation rate, surface referent

<sup>a</sup> Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RM. 1987. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J Bone Miner Res* 2:595-610.

Measurements of static and dynamic parameters in distal radius (Table G3):

### Cortical bone:

Effect of OVX (relative to sham):

- Significant increase in MS/Md.S, MAR, BFR/Md.S, BFR/Md.V, BFR/TV, Ps.MS/Md.S, Ps.BFR/Md.S
- No effect on Md.V/TV, Md.S/Md.V

### What this means:

OVX caused an increase in intracortical and periosteal bone formation, without consequence for bone surface or volume density (i.e. increased turnover but resorption balances formation)

Effect of LY treatment (relative to OVX):

- Significant increase in Md.S/Md.V (BS/BV),
- N.s. increase in BFR/Md.V, BFR/TV, Ps.MS/Md.S, Ps.BFR/Md.S
- Small but n.s. decrease in Md.V/TV

### What this means:

PTH caused increased intracortical and periosteal bone formation, with consequently increased intracortical bone surface and a small effect on bone volume density.

Effect of LY treatment withdrawal (relative to OVX):

- Some parameters were reversed to OVX levels, but were not significantly different from OVX (i.e., Md.S/Md.V, MS/Md.S, BFR/Md.V, BFR/TV, Ps.MS/Md.S, Ps.BFR/Md.S)
- No effect on Md.V/TV

### What this means:

PTH withdrawal decreased cortical and periosteal bone formation to OVX or below-OVX levels. Withdrawal reversed the increase in bone surface density. Bone volume density remained unaffected.

Conclusion: Intracortical and periosteal bone formation were increased by ovariectomy, and further increased by PTH treatment, and this appeared to cause a small decrease on cortical bone volume density. Bone formation parameters were reversed by PTH withdrawal.

### Cancellous bone:

Effect of OVX (relative to sham):

- Significant increase in MS/Md.S, MAR, BFR/Md.S, BFR/Md.V, BFR/TV
- N.s. increase in Tb.Sp
- N.s. decrease in Tb.N.
- No effect on Md.V/TV, Md.S/Md.V, Tb.Th

What this means:

OVX caused an increase in bone formation (turnover) without a significant effect on trabecular bone parameters or cancellous bone volume or surface density.

Effect of LY treatment (relative to OVX):

- Significant increase in Md.V/TV, Tb.N
- Significant decrease in Tb.Sp
- N.s. increase in MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV
- No effect on Md.S/Md.V, Tb.Th

What this means:

PTH caused an increase in cancellous bone formation with consequently increased bone volume density and improved trabecular parameters.

Effect of LY treatment withdrawal (relative to OVX):

- Some parameters were reversed to or below OVX levels, but were not significantly different from OVX (i.e., Md.V/TV, Tb.N, Tb.Sp, MS/Md.S, BFR/Md.S, BFR/Md.V, BFR/TV)

What this means:

PTH withdrawal caused a decrease in bone formation to OVX or below-OVX levels. Withdrawal partially reversed bone volume and trabecular parameters.

Conclusion: Cancellous bone formation but not bone volume was increased by OVX, whereas both bone formation and bone volume were increased by PTH treatment. Bone formation, volume density and trabecular parameters were reversed by PTH withdrawal.

**Table G3 Distal Radius Data n=119 (Mean + SEM of Raw Data) and Bonferroni-Adjusted Group Comparisons (121 Monkey Population)**

Parameter	Tissue	Sham n = 21*		OVX n = 20		PTH1 n = 18		PTH1-W n = 20*		PTH5 n = 21		PTH5-W n = 19		Trans-formation	Over-all p	Bonferroni-Adjusted p			
		Sham	OVX	PTH1	PTH1-W	PTH5	PTH5-W	Sham	OVX vs PTH1	OVX vs PTH1-W	OVX vs PTH5	OVX vs PTH5-W							
MDV/TV	Cortical	56.0 ± 1.7	61.7 ± 2.2	57.2 ± 2.3	58.0 ± 1.9	57.5 ± 1.8	56.9 ± 2.8	12	ns	ns	ns	ns	ns						
	Cancellous	17.1 ± 1.0	16.1 ± 0.9	19.7 ± 1.2	19.3 ± 1.1	22.1 ± 1.2	18.4 ± 1.1	0	ns	ns	ns	ns	ns						
MS/MdV	Cortical	8.7 ± 0.7	8.1 ± 0.6	12.4 ± 0.8	9.5 ± 0.8	11.0 ± 0.8	9.9 ± 0.9	square root	0.0	ns	<.0005	ns	<.05						
	Cancellous	27.2 ± 1.1	24.7 ± 1.3	26.9 ± 1.2	25.2 ± 0.9	24.1 ± 1.2	25.3 ± 1.2	log	25	ns	ns	ns	ns						
Tb.Th	Cancellous	74.8 ± 2.7	84.8 ± 4.1	77.0 ± 3.6	81.1 ± 3	86.1 ± 3.3	83.0 ± 4.6		22	ns	ns	ns	ns						
	Cancellous	2.3 ± 0.1	1.9 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	c	0.0	ns	<.01	<.05	<.01						
Tb.Sp	Cancellous	391 ± 25.7	471 ± 39	332 ± 25.6	363 ± 26	318 ± 16.6	380 ± 25		0.0	ns	<.01	<.05	<.01						
	Cancellous	8.7 ± 0.2	32.6 ± 2.9	30.2 ± 2.3	29.0 ± 3.8	32.5 ± 2.6	25.6 ± 3.3	c	0.0	<.01	ns	ns	ns						
MAR	Cortical	11.1 ± 2.6	29.1 ± 3.3	31.1 ± 2.4	26.3 ± 4	30.3 ± 2.3	24.3 ± 3.3	c	0.0	<.01	ns	ns	ns						
	Cancellous	0.5 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	c	0.0	<.01	ns	ns	ns						
BFR/MdS	Cortical	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0		0.1	<.01	ns	ns	ns						
	Cancellous	18.7 ± 4.8	98.7 ± 9.7	72.2 ± 6.7	78.6 ± 10.7	80.7 ± 8.1	70.0 ± 10.3	c	0.0	<.01	ns	ns	ns						
BFR/MdV	Cortical	22.0 ± 5.2	67.1 ± 8.3	63.9 ± 6.2	59.5 ± 9	61.2 ± 5.6	52.6 ± 7.6	c	0.0	<.01	ns	ns	ns						
	Cancellous	16.2 ± 4.2	68.4 ± 7.7	67.1 ± 9.2	69.9 ± 9.7	69.8 ± 11.1	61.2 ± 8.5	c	0.0	<.01	ns	ns	ns						
BFR/TV	Cortical	55 ± 12.2	192 ± 18.7	178 ± 17.6	165 ± 22	193 ± 15	123 ± 15.6	c	0.0	<.01	ns	ns	ns						
	Cancellous	8.8 ± 2.3	41.9 ± 4.5	45.4 ± 4.5	41.6 ± 5.8	49.7 ± 5.7	35.6 ± 5.1	c	0.0	<.01	ns	ns	ns						
P-MdS	Cortical	9.0 ± 2.0	25.4 ± 3.2	33.3 ± 3.9	27.8 ± 4	41.9 ± 3.5	23.1 ± 3.4	square root	0.0	<.0005	ns	ns	<.05						
	Cancellous	7.9 ± 2.0	24.5 ± 3.1	29.1 ± 3.9	16.8 ± 2.3	29.6 ± 2.7	20.9 ± 3.0		0.0	<.0005	ns	ns	ns						
P-MAR	Cortical	0.5 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	c	0.0	<.01	ns	ns	ns						
	Cancellous	16.2 ± 5.2	82.0 ± 9.0	66.6 ± 9.2	35.8 ± 6.8	58.9 ± 6.9	41.9 ± 6.6	c	0.0	<.01	ns	ns	ns						

ns = p > .1.

\* Exception: P-MAR (n=15)

\* Exception: P-MAR (n=19)

\* Data not amenable to transformation, analyzed using Kruskal-Wallis test.

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### Three-Dimensional Modeling of the Effects of Recombinant PTH(1-34) on the Bone Distribution of Lumbar Vertebra from Cynomolgus Monkeys

(Non-Clinical Pharmacology Report BN5-08)

A sophisticated 3D histomorphometry study was carried out of lumbar vertebrae, using high resolution QCT and computerized modeling techniques to estimate voxel (bone element) BMD distribution and effective vertebral strain.

#### Methods

Test animals: 6 groups (sham control, OVX, PTH5, PTH5W), N=7/group.

QCT: BMC, X-area, BV/TV, TbTh, TbN, TbSp, connectivity

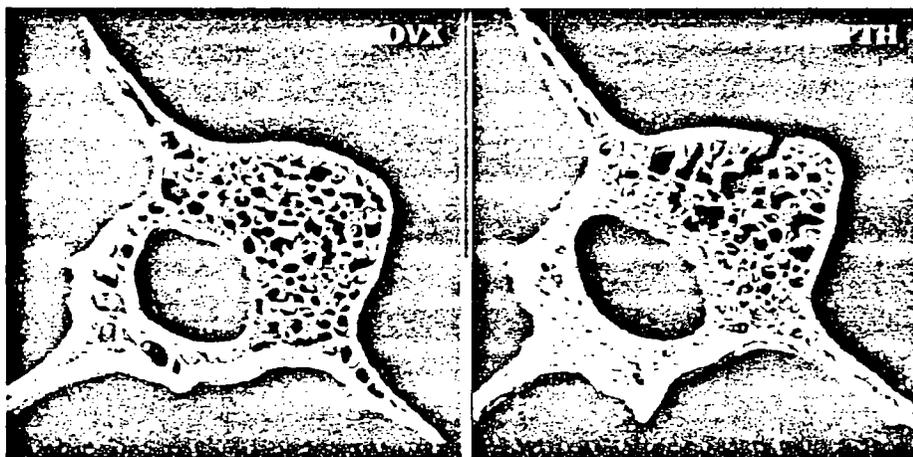
Scanning: Voxels, modeled into tetrahedral finite elements (490x490x500  $\mu\text{m}$ )

Calculation: BMD and Young's modulus for each bone element, determining density distribution

Analysis: Linear elastic stress analysis (compression), determining vertebral stress and strain distribution

#### Results

QCT: Bone mass



**Figure 1** Representative scans of monkey L-5 vertebra. Excised L-5 were scanned serially by quantitative computed tomography (QCT), using 70 x 70 x 500  $\mu\text{m}$  voxels. Middle slices of OVX and PTH5 are shown as examples.

- L5 cross section (500  $\mu\text{m}$  middle slice, Fig.1): Small n.s. decrease in BMD by OVX, 21% increase relative to OVX by PTH5
- L5 midsagittal section (centrum): Small n.s. decrease in BMD by OVX, 30% increase in BMD by PTH5 relative to OVX, 7% decrease in BMD by PTH5W relative to PTH5
- Bone area and volume not affected (not shown)

Histomorphometry (by QCT) (middle slice in cross-section)

- Significant decrease of BV/TV and TbN, increase in TbSp, and decrease in connectivity by OVX
- Significant increase in structural parameters BV/TV, Tb.Th, Tb/N, decrease in Tb.Sp, and increase in connectivity parameters (node density) by PTH5
- No effect on any parameter of PTH5W relative to PTH5

Neural canal/histology

- Area of neural canal cross sectional area not affected by OVX or PTH.
- Bone response of PTH mainly located along endocortical and trabecular but not periosteal surface

Density distribution of bone elements (voxels) (Fig.4)

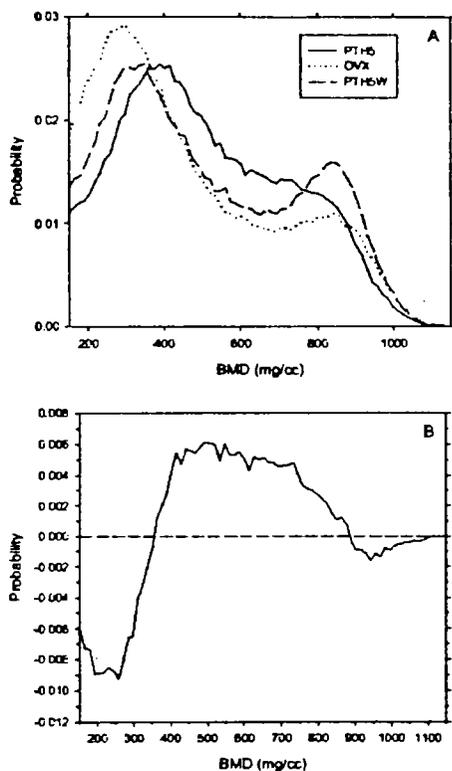
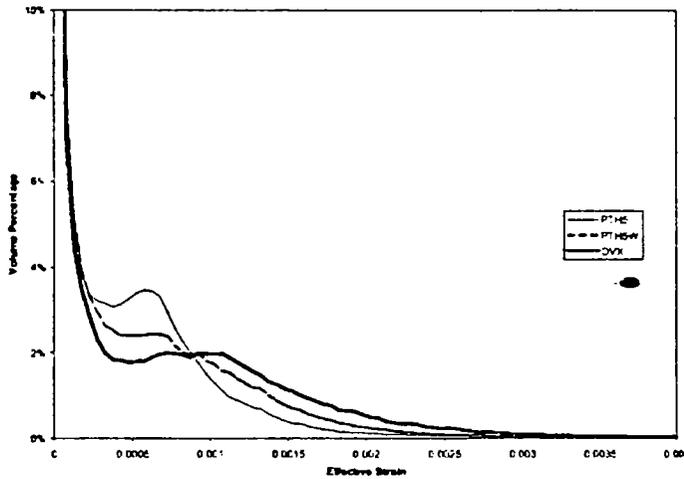


Figure 4

Histogram analysis of BMD for whole L-5 vertebra. BMD distributions were pooled and are shown for PTH5, OVX, and PTH5W vertebra (A). A bimodal distribution of voxels ( $490 \times 490 \times 500 \mu\text{m}$ ) was observed. A difference histogram was generated to highlight differences between PTH5 and OVX (B). These histograms show that PTH5 reduced the amount of low density bone by increasing the amount of medium density bone, compared to OVX. Withdrawal of PTH for 6 months (PTH5W) induced a redistribution of medium density bone into lower and higher density bone, with the result that PTH5W had less low density and more high density bone than OVX.

- No effect of OVX
- Decrease in proportion of low density voxels and increase in medium density voxels by PTH5 (shift of low density bone to medium density bone). Thus, PTH appeared to convert low density bone into medium-density bone.
- Decrease in medium density voxels and increase in both low- and high density voxels by PTH5W (relative to PTH5). Result was that there were more high density elements in PTH5W than in PTH5 and OVX groups.
- No hypermineralization (BMD range of PTH5 similar to sham or OVX)

Strain distribution (centrum, mid-sagittal) (Fig. 7)



**Figure 7** Analysis of the effective strain distribution for PTH5, PTH5W, and OVX Vertebra (Centrum) loaded in compression. The strain histograms show that fewer bone elements in PTH5 vertebra experience strain greater than 0.001 compared to OVX. The average strain for PTH5 was 36% less than OVX. The strain histogram for PTH5W was intermediate between PTH5 and OVX with average strain 23% below OVX.

- Reduction of average strain by PTH5. Reduction bone elements under high strain ( $>0.0008$ ) by PTH5 relative to OVX, and increase of bone elements under moderate strain ( $0.0002-0.0008$ ) by PTH5 relative to OVX. This indicates a greater resistance to failure for vertebrae loaded in compression.
- Distribution in PTH5W group intermediate between OVX and PTH5

**Note:**

The previous compression analysis results of LV3-LV4 (Study X-95-11) are in accordance with the strain distribution results obtained in this study. Shift to high (and low) density bone elements in PTH5W group maybe due to relatively large unstability of mid-density elements (e.g. some of these elements maybe porous cortical bone since PTH increases porosity, and these may fill up after PTH withdrawal).

**Conclusions**

PTH treatment caused micro-structural bone changes in vertebra which were accompanied by increased strength (decreased likelihood to fail) in both PTH5 and in PTH5W groups relative to OVX.

**Serum Pharmacokinetics of LY333334 in Adult Ovariectomized Female Cynomolgus Monkeys Following a Daily Subcutaneous Dose of 1 or 5 ug/kg LY333334 for 17 Months (ADME Report 15)**

**Methods**

Serum pharmacokinetics of immunoreactive LY333334 in adult ovariectomized (OVX) female cynomolgus monkeys following daily subcutaneous dosing of 1 or 5 µg/kg for 1,7,11, or 17 months was reported. Serum concentrations of immunoreactive LY333334 were determined by a validated immunoradiometric assay (IRMA) in samples collected at 0, 0.17, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, and 4 hours after dosing, in the PTH1 and PTH5 groups.

**Results**

The serum concentrations of immunoreactive LY333334 in nearly all monkeys prior to dosing were determined to be BQL. Most of the serum levels were also BQL 3 hours postdose for 1 µg/kg or 4 hours postdose for 5 µg/kg.

For the 1 ug/kg/d group, with regard to proportion of BQL values a satisfactory time interval was established to be from 0 to 1 hour for the AUC<sub>0-t</sub> calculation. For the 5 ug/kg/d group, the time interval from 0 to 2 hours was established for the AUC<sub>0-t</sub> calculation.

**Table 11 Estimated Mean AUC<sub>0-t</sub> for Each Dose Group and Month**

Dose (µg/kg)		1 month AUC <sub>0-t</sub> <sup>(1)</sup> (ng·mL·hr)	7 month AUC <sub>0-t</sub> (ng·mL·hr)	11 month AUC <sub>0-t</sub> (ng·mL·hr)	17 month AUC <sub>0-t</sub> (ng·mL·hr)
1	Mean	0.25	0.46	0.29	0.24
	SE <sup>(2)</sup>	0.06	0.22	0.03	0.02
	N <sup>(3)</sup>	15	14	13	13
5	Mean	2.03	2.53	2.45	1.85
	SE	0.31	0.28	0.15	0.18
	N	13	16	14	16

<sup>(1)</sup> AUC<sub>0-t</sub> = AUC from 0 to t where t=1 hour for the 1 µg/kg group and t=2 hours for the 5 µg/kg group.

<sup>(2)</sup> SE = standard error.

<sup>(3)</sup> N = total number of animals used in the sparse sampling scheme.

Note: AUC was increased more than proportionally with dose in the range of 1 ug/kg/d to 5 ug/kg/d. This was also seen in a 2-year rat carcinogenicity study, in the dose range of 5-30 ug/kg/day, which is associated with an AUC range (0.5-7.5 g/mL·hr) similar to the one observed in this monkey study.

The data indicated:

- There was no accumulation of LY333334 after 17 months of once daily subcutaneous dosing.
- There was no time dependent trends in mean AUC for either the 1 or 5 µg/kg dose group, suggesting unchanged exposure upon repeated dosing for 17 months.

**Human exposure multiples**

Human exposure multiples in 18-month monkey study with doses of 1 and 5 ug/kg/day

Dose (ug/kg/day)	Monkey AUC of PTH(1-34) (ngxhr/ml) (Month 11)	Monkey AUC of PTH(1-34) (ngxhr/ml) (Month 17)	AUC multiples (monkey:human) (Month 11)*	AUC multiples (monkey:human) (Month 17)*
1	0.29	0.24	0.98x	0.81x
5	2.45	1.85	8.3x	6.3x

\*Human AUC=0.295 ngxhr/ml (median value; dose 20 ug/day; 0.3 ug/kg/day; Clinical trial GHAC)

**Conclusion**

Exposure in the PTH1 group was similar to expected human exposure, while exposure in the PTH5 group was approximately 7 times the expected human exposure.

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**Histopathologic Evaluation of Kidneys From Ovariectomized Female Cynomolgus Monkeys Given LY333334 Daily by Subcutaneous Injection for up to 18 Months (Study P03196)**  
(Toxicology Report 10)

Ovariectomized cynomolgus monkeys were given LY333334 doses of 1 or 5 µg/kg for 18 months or for 12 months with a 6-month withdrawal period. At necropsy, kidneys were collected and submitted to Lilly Research Laboratories for histologic processing and evaluation.

There were no gross findings in control or treated animals. A variety of renal histopathologic lesions were present in control and treated groups. The most frequent finding was subacute multifocal inflammation which occurred in almost all animals.

**Conclusion**

LY333334 had no gross or histopathologic effects in the kidneys of OVX monkeys treated for 12 or 18 months. The no-observed-effect level (NOEL) for renal histologic changes was  $\geq 5$  µg/kg/day.

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## **SUMMARY AND EVALUATION OF MONKEY BONE QUALITY STUDY**

In this long term bone quality study, mature ovariectomized cynomolgus monkeys were treated with LY333334 (1 or 5 ug/kg/day) for 18 months, or for 12 months with a subsequent 6-month period in which treatment was withdrawn. Results were compared with those from sham-operated untreated animals.

The PK of LY333334 in the OVX animals did not appear to change over time. There was no drug accumulation over the course of the treatment period, and the AUC values for the 1 and 5 ug/kg/day groups were approximately 0.3 and 2.2 ngxh/ml, respectively. This represents human exposure multiples of approximately 1x and 7x the expected exposure at the intended clinical dose of 20 ug/day.

After 18 months, effects of LY333334 on serum biochemistry included decreases in serum phosphorus and endogenous PTH, increases in serum calcitriol and urinary cAMP/creatinine ratio. There were no effects on serum Ca (total or ionized), serum creatinine or serum BUN. Assessment of bone turnover markers showed small, although not statistically significant increases in serum alkaline phosphatase, osteocalcin, and urinary C-terminal collagen fragments (Crosslaps). PTH at 1 or 5 ug/kg/day had no significant histopathology effects in the ovariectomized monkey.

### **Bone mass**

Ovariectomy resulted in a relative osteopenia, as the intact animals gained bone mass, while the ovariectomized ones remained stable or gained less bone mass than the sham animals.

In the spine, LY333334 caused a gradual dose-dependent increase in projected BMD ( $\text{mg}/\text{cm}^2$ ). At 18 months, the BMD in both PTH treatment groups (PTH1 and PTH5) was larger than the BMD of the sham animals. PTH also increased the projected area ( $\text{cm}^2$ ) of the spine in the high dose group. In the whole body, PTH caused a dose-dependent increase in projected BMD with no significant effects on projected area.

In the midshaft of the radius there was a decrease in volumetric BMD ( $\text{mg}/\text{cm}^3$ ) in both PTH groups, and a dose-dependent increase in cross-sectional area (X-area,  $\text{mm}^2$ ).

In the proximal tibia, there were marked dose-dependent increases in BMD ( $\text{mg}/\text{cm}^3$ ) of the innermost cancellous zone and the two intermediate zones. In the outer, cortical zone BMD was not significantly affected by PTH. There was no effect on X-area.

In the distal radius, a small decrease in BMD was observed in the outer cortical zone and a dose-dependent increase was seen in the innermost cancellous zone. There were no clear effects on the BMD of the intermediate zones. There was no effect on X-area.

Generally, the effect of LY333334 withdrawal on bone mass consisted of either no or a small reversal after withdrawal of the low dose (PTH1W), and a significant reversal after withdrawal of the high dose (PTH5W).

### **Bone strength**

In the lumbar vertebrae (L3 and L4 average data) LY333334 caused a dose-dependent increase in yield strength, yield stress, and Young's modulus, so that the values for these parameters in the PTH5 group were larger than in the sham group. Spinal BMD ( $\text{g}/\text{cm}^3$ ) was significantly related to vertebral yield strength ( $r=0.83$ ), and the correlation appeared to be similar for all treatment groups including sham and OVX.

Data from a high resolution QCT study showed that PTH treatment caused micro-structural bone changes in vertebra which were accompanied by increased strength (decreased likelihood to fail) in both PTH5 and in PTH5W groups relative to OVX.

In the humerus midshaft, ultimate force, stiffness and work to failure were slightly but not significantly decreased by OVX and increased dose-dependently although not significantly by LY333334. The

humeral midshaft cortical thickness was increased by LY333334, significantly so in the PTH5 group. The effect on ultimate force ( $F_u$ ) is likely to be related to the effect on thickness, which is presumed to offset an increase in cortical porosity by PTH (see histomorphometry results below).

In the femoral neck ultimate force ( $F_u$ ) was significantly decreased in the OVX group, and significantly and dose-dependently increased in both PTH groups.

In femoral midshaft beam specimens whose properties are not dependent on bone geometry, ultimate stress appeared to be decreased in the PTH5 group. Young's modulus was slightly decreased in the OVX group, and further decreased by LY333334, so that this parameter was significantly lower in PTH5 groups than in the sham group. The data indicated a drug-related decrease in intrinsic stiffness and a concomitant decrease in ultimate stress of the cortical bone material. As in the humerus, this may have been due to an increase in cortical porosity (see histomorphometry data below).

Generally, the effects of LY333334 withdrawal on bone strength were inconsistent. In some instances, there was a reversal of the effects of PTH, and in other cases there was no difference between treatment and withdrawal groups. This appeared to depend on the bone site evaluated and the dose of PTH used.

#### Histomorphometry

In the iliac crest, LY333334 increased bone formation and either decreased or increased bone resorption depending on the duration of treatment. Regardless of the effect on resorption, treatment with PTH resulted in a significant increase in cancellous trabecular bone. LY333334 also appeared to induce a small increase in wall width (bone balance). LY333334 caused an increase in tunneling and a transient increase in cortical thickness with levels actually decreased below OVX after 15 months. The increase in tunneling was also observed in vertebral trabeculae. Cortical porosity in the iliac crest was not affected.

In the humerus midshaft, LY333334 increased endocortical but not periosteal bone formation, which resulted in an increase in cortical area. LY333334 at both doses increased cortical porosity, apparently due to increased intracortical resorption.

The histomorphometry and biomechanical strength data for the humerus midshaft suggest that increased cortical haversian remodeling and increased cortical porosity induced by PTH do not necessarily have deleterious effects on bone strength. This is due in part to the porosity being mainly located in the endocortical zone where it exerts relatively small effects on bone strength, and the fact that the increased porosity can be accompanied by a PTH-induced increase in cortical thickness due to a stimulation of cortical bone apposition.

However, an increase in porosity (decrease in BMD) may not be offset by an increase in thickness at all cortical bone sites. Thus, the long term cortical bone effects and safety remain a concern for PTH treatment.

Histomorphometry was also carried out of the mid-radius, the mid-femur, the vertebrae, the femoral neck and the distal radius. Although at all bone sites PTH increased bone formation, the effect of this increase on the structure of the bone varied with the different bone sites and type of bone (cortical or cancellous). Generally, the increased bone turnover/formation induced by PTH at cortical bone sites caused increased porosity and either a decrease (mid-radius, distal radius cortex), increase (vertebral cortex), or no effect (femoral shaft, femoral neck cortex) on fractional bone volume (BV/TV).

The anatomical site of the increased cortical bone formation (periosteal, endocortical, intracortical) and the effect of PTH on cortical bone area or thickness depended on the bone site under evaluation. Bone area or thickness was increased in humerus and possibly midradius and vertebrae, while it was unchanged in midfemur and distal radius. The latter data are from either densitometric or histomorphometric assessments.

By contrast, the increased bone formation induced by PTH in cancellous bone always resulted in an increase in cancellous bone volume and improved trabecular architecture (more, thicker, and less separated trabeculae).

The effect of PTH withdrawal always consisted of a decrease in bone formation rate and related parameters, sometimes to levels below those in the OVX group (particularly after PTH5 withdrawal). Moreover, if effects on fractional bone volume (BV/TV) had been observed with PTH treatment (e.g. in vertebrae, cancellous bone of femoral neck and distal radius), withdrawal would usually reverse these effects, although this did not happen in the midradius (and perhaps the cortical bone of the distal radius) where PTH caused a decrease in this parameter and withdrawal did not reverse that effect.

The predictive value of the preclinical monkey study data was generally supported by the BMD and fracture data obtained in the pivotal clinical trial B3D-MC-GHAC (17-23 month study in postmenopausal women). In trial GHAC, after ca. 21 months of treatment with 20 ug/day, BMD was markedly increased at the lumbar spine (9% as compared to placebo), and the risk of one or more new vertebral fractures was significantly decreased (by 65%). The BMD was also increased albeit less pronounced at sites with a relative larger contribution of cortical bone (hip, femoral neck, intertrochanter area and Ward's triangle). However, in the distal radius BMD was decreased as compared to placebo (average -1% at 20 ug, -2% at 40 ug). The decrease was larger in the radial midshaft (mainly cortical bone), and was statistically significant at this site at the 40 ug/day dose. Further pQCT evaluation of the radius (proximal area) indicated that periosteal and endosteal circumferences and total bone area were increased while BMC was unchanged, which would reduce BMD but increase computed measures of bone strength. The latter appears to be analogous to the effects of LY333334 in the monkey humerus and radius.

The potential implication of the preclinical data that cortical bone strength might be impaired so far has not been borne out by clinical fracture data. The incidence of fragility fractures at nonvertebral sites (hip, wrist, ankle, humerus, rib, foot, pelvis) was either unaffected (humerus, foot) or decreased (other sites) in trial GHAC. However, the number of fractures was too small to demonstrate a statistically significant fracture risk reduction at individual nonvertebral sites. Transiently increased cortical porosity in the human iliac crest confirms that this phenomenon also takes place in humans.

The decrease in clinical non-vertebral fractures may be due to the positive effect of LY333334 on cancellous bone which is present at most non-vertebral sites (e.g. femoral neck/hip), combined with a generally positive effect on cortical bone thickness or area and a relatively small adverse effect of an increase in porosity. Thus, although the small numbers of non-vertebral fractures preclude a solid conclusion, the clinical data suggest that there is no major safety problem in cortical bone.

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## RABBIT BONE QUALITY STUDIES

Two studies were conducted in intact rabbits, to evaluate the effects of LY333334 on cortical bone properties. Unlike the rat, the rabbit is a species in which there is osteonal (Haversian, cortical) bone remodeling, as in monkey and human. The remodeling period in the rabbit is ca. 70 days, compared to ca. 90 days in primates.

Report	Title	In Vivo		In Vitro		Results
		Species, Strain, Gender, & Age	Dose Route	Assay Type	Tissue/ Cell Line	
CG3-06	Histomorphometric and Biomechanical Analyses of the Anabolic Effects of LY333334 on the Cortical Bone of Ovary-Intact Adult Rabbits	Rabbit, New Zealand, ovary-intact female, mature	10 & 40 µg/kg/day SC once daily for 140 days			LY333334 increased biomechanical measures of strength in rabbit cortical bone by restructuring bone architecture via appositional bone formation, favorable geometric changes, & replacement of pre-existing matrix with new matrix due to increased activation frequency in long bones. The high dose was associated with a transient increase in serum calcium which remained within physiologic range.
CG3-13	Effects of Human PTH (1-34), LY333334, on Bone Mass, Remodeling, and Mechanical Properties of Cortical Bone During the First Remodeling Cycle in Rabbits	Rabbit, New Zealand, ovary-intact female, mature	10 µg/kg/day SC once daily for 35 (P35), 70 (P70), or 140 (P140) days			Cortical bone histomorphometry of tibial mid-shaft showed intracortical remodeling was significantly increased by PTH without significant increase of cortical porosity in first remodeling cycle. This was associated with significantly greater cortical area & bone strength in P35 & P70 groups due to stimulation of periosteal bone formation rate. By 140 days, surface remodeling effects declined so there was no difference between control & treated groups. PTH increased cancellous bone volume & biomechanical properties in 3 <sup>rd</sup> lumbar vertebra. Although intracortical remodeling increases within first remodeling period of 70 days, the greater cortical area due to concomitant periosteal expansion increases cortical bone strength.

### Histomorphometric and Biomechanical Analyses of the Anabolic Effects of LY333334 on the Cortical Bone of Ovary-Intact Adult Rabbits

(Nonclinical Pharmacology Report CG3-06)

*Note: The results of this study were published by Hirano et al. (J. Bone Min. Res., Vol. 14, #4, p536) (1999)*

#### METHODS

Female, ovary-intact New Zealand white rabbits (n=6/group), approximately 9 months old, weighing 3.25 to 3.75 kg, were treated with LY333334 (10 or 40 µg/kg/day, PTH10 or PTH40) or vehicle by subcutaneous injection, once daily, on 5 days per week, for 140 days (approximately 2 remodeling cycles). Bone mass data (pQCT or pDEXA) were collected on proximal femur, femur mid-shaft, and lumbar vertebra, LV4. Biomechanical assays of strength were done on femur mid-shaft and lumbar vertebra, LV5. Intrinsic material properties were assessed by acoustic microscopy on the humerus mid-shaft. Histomorphometry was done on tibia mid-shaft and lumbar vertebra, LV3.

#### RESULTS

Body weight gain was decreased in treated groups, and there was a slight loss of body weight at PTH40. At 4h after the last injection, a small increase of 1.4 mg/dL in serum calcium was observed in the PTH40 group. Serum alkaline phosphatase increased by 2-fold in the PTH40 group. Serum phosphate did not vary significantly between groups.

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In the cortical mid-shaft of the femur, bone mineral density (BMD), bone mineral content (BMC), and cross-sectional bone area (X-area) were increased by PTH. In the proximal femur, BMD and BMC, but not X-area, were increased dose-dependently. In LV4 there were no effects of PTH on BMD, BMC or X-area (Table 3).

**Table 3** Bone Mass and Geometry of Lumbar Vertebra, LV4, Proximal Femur and Femur Mid-Shaft in Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily, for 5 Months<sup>a</sup>

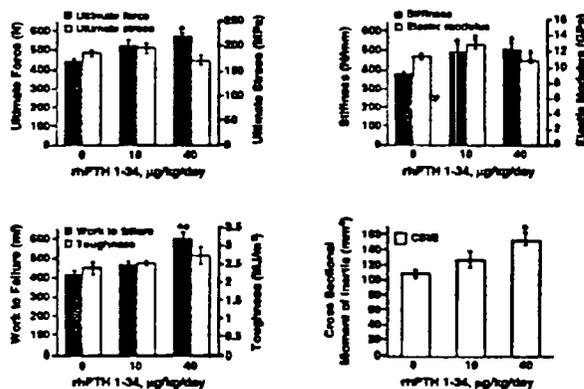
	Control	PTH10	PTH40
<b>Vertebra LV4</b>			
Bone mineral density vBMD, mg/cm <sup>3</sup>	482 ± 34	465 ± 20	463 ± 26
Bone mineral content BMC, mg	53 ± 2	54 ± 3	54 ± 3
Cross-sectional area XSA, mm <sup>2</sup>	98 ± 10	97 ± 3	97 ± 2
Voxels	1130 ± 116	1114 ± 32	1118 ± 26
<b>Proximal Femur</b>			
Bone mineral density aBMD, g/cm <sup>2</sup>	0.251 ± 0.002	0.285 ± 0.009 <sup>b</sup>	0.305 ± 0.009 <sup>b</sup>
Bone mineral content BMC, g	0.99 ± 0.02	1.12 ± 0.04 <sup>b</sup>	1.21 ± 0.04 <sup>b</sup>
Cross-sectional area XSA, mm <sup>2</sup>	3.94 ± 0.09	3.92 ± 0.05	3.99 ± 0.12
<b>Mid femur</b>			
Bone mineral density vBMD, mg/cm <sup>3</sup>	929 ± 21	989 ± 20	1051 ± 19 <sup>b</sup>
Bone mineral content BMC, mg	53 ± 2	60 ± 2 <sup>b,c</sup>	70 ± 2 <sup>b</sup>
Cross-sectional area XSA, mm <sup>2</sup>	48 ± 2	51 ± 2	55 ± 1 <sup>b</sup>
Voxels	2210 ± 100	2333 ± 87	2532 ± 58 <sup>b</sup>

<sup>a</sup> Data are expressed as mean ± SEM for 6 rabbits per group, except for total bone mineral content for LV5 where n=5

<sup>b</sup> p < .05 compared with control.

<sup>c</sup> p < .05 compared with PTH(1-34) 40 µg/kg/day.

In the femoral midshaft, bone strength measured as ultimate force, stiffness, work to failure and apparent cross-sectional moment of inertia (CSMI) was increased by PTH and all parameters were significantly increased in the PTH40 group. Young's modulus was significantly lower in the PTH40 than in the PTH10 group but was not different from controls (Fig. 1).



**Figure 1**

Biomechanical data obtained from *in vitro* strength testing by 3-point bending of the femur diaphyseal mid-shaft of ovary-intact rabbits, given vehicle (0) or PTH once daily for 5 months. Statistical significance, p < .05, \* vs control (0); # PTH at 10 µg/kg vs 40 µg/kg.

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In the lumbar vertebrae, there were no significant effects of PTH on biomechanical properties (Table 4).

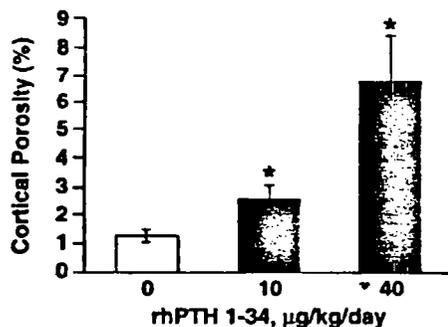
**Table 4** Biomechanical Measures of Strength in Lumbar Vertebra, LV5, of Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10  $\mu\text{g}/\text{kg}$  (PTH10) or 40  $\mu\text{g}/\text{kg}$  (PTH40) Once Daily for 5 Months<sup>a</sup>

	Control	PTH10	PTH40
<b>Vertebra LV5</b>			
Ultimate force (Fu, N)	1539 $\pm$ 112	1653 $\pm$ 72	1552 $\pm$ 92
Stiffness (S, slope, N/mm)	5684 $\pm$ 409	5705 $\pm$ 433	5670 $\pm$ 384
Cortical thickness (t, $\mu$ )	10.0 $\pm$ 0.5	9.8 $\pm$ 0.3	10.4 $\pm$ 0.4
Cross-sectional area (XSA, mm <sup>2</sup> )	67 $\pm$ 3	65 $\pm$ 3	68 $\pm$ 3
Ultimate stress ( $\sigma_u$ , Mpa)	23 $\pm$ 1	26 $\pm$ 2	23 $\pm$ 1
Young's modulus (E)	861 $\pm$ 90	856 $\pm$ 55	872 $\pm$ 65
Toughness (u, MJ m <sup>-3</sup> )	0.46 $\pm$ 0.05	0.58 $\pm$ 0.07	0.45 $\pm$ 0.03

<sup>a</sup> Data are expressed as mean  $\pm$  SEM for 6 rabbits per group except for total bone mineral content for LV5 where n=5. No statistically significant differences were detected between groups.

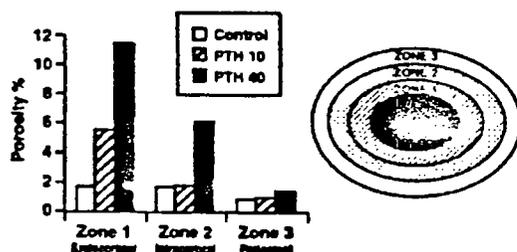
PTH did not alter intrinsic material properties of cortical bone (humerus intrinsic stiffness).

Histomorphometry was carried out in tibial midshaft and lumbar vertebrae. In the cortical bone of the tibial mid-shaft LY caused a marked dose-dependent increase in intracortical activation frequency (AcF) (Table 6). LY also caused a dose-dependent increase in cortical porosity (Fig. 2). The increased porosity in both PTH10 and PTH40 groups was predominantly in the endocortical (Zone 1) region (Fig. 3). The increased porosity probably reflects the "birth" of multiple new sites of bone turnover.



**Figure 2**

Cortical porosity in histologic cross-sections of the tibial mid-shaft of ovary-intact female rabbits given vehicle (0) or PTH once daily for 5 months. Statistical significance,  $p < 0.05$ . \* vs control.



**Figure 3** Spatial localization of cortical porosities into one of three concentric zones, arbitrarily superimposed on histologic cross-sections of the tibial mid-shaft of rabbits given vehicle or PTH once daily for 5 months. Note that porosities preferentially cluster within the endocortical area (Zone 1) of cortical bone. Because of this localization, minimal effect may be expected on cross-sectional moment of inertia (CSMI). As shown in Figure 1, CSMI, a measure of strength, increased due to increased surface bone formation on endosteal and periosteal surfaces.

In the tibial midshaft, LY increased bone formation on periosteal (PsMS/BS, PsBFR/BS) and endocortical (EcMS/BS, EcBFR/BS) surfaces, in either low and high dose or only high dose groups (Table 5). Intracortically, the number of resorption sites (RsN/CtAr) was increased in the PTH40 group, and bone formation rate (BFR/BV) was increased at both doses (Table 6). Bone area (B.Ar=total area within periosteal surface including marrow area) was increased in the PTH40 group, and marrow area (Ma.Ar) was slightly but not significantly decreased by PTH. Cortical area (Ct.Ar=area of bone within periosteal surface including porosities) and %cortical area (%CtAr=Ct.Ar/B.Ar) were significantly increased in both groups.

**Table 5** Periosteal and Endocortical Bone Remodeling of Tibial Mid-Shaft in Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily for 5 Months<sup>a</sup>

Parameter	Abbreviations	Control	PTH10 <sup>b</sup>	PTH40 <sup>c</sup>
Endocortical osteoid surface	EcOS/BS (%)	8.8 ± 6.0	13.7 ± 10.5	20.2 ± 5.8
Endocortical osteoid thickness	EcOTh (µm)	7.4 ± 2.4	3.7 ± 2.3	8.1 ± 0.9
Periosteal mineral apposition rate	PsMAR (µm/day)	0.35 ± 0.17	0.38 ± 0.08	0.66 ± 0.14
Endocortical mineral apposition rate	EcMAR (µm/day)	1.33 ± 0.22	0.79 ± 0.16	1.32 ± 0.15
Periosteal mineralizing surface	PsMS/BS (%)	3.8 ± 1.9	8.2 ± 2.1	22.3 ± 2.7 <sup>c</sup>
Endocortical mineralizing surface	EcMS/BS (%)	26.4 ± 6.6	32.6 ± 8.2	57.7 ± 10.4 <sup>b</sup>
Periosteal bone formation rate	PsBFR/BS (µm <sup>3</sup> /µm <sup>2</sup> -yr)	0.02 ± 0.02	0.03 ± 0.01	0.16 ± 0.05 <sup>b,c</sup>
Endocortical bone formation rate	EcBFR/BS (µm <sup>3</sup> /µm <sup>2</sup> -yr)	0.04 ± 0.10	0.31 ± 0.10	0.72 ± 0.12 <sup>b,c</sup>

<sup>a</sup> Data are expressed as mean ± SEM for 6 rabbits per group.

<sup>b</sup> p < 0.05 compared with control.

<sup>c</sup> p < 0.05 compared with PTH(1-34) 10 µg/kg/day.

**Table 6** Intracortical Bone Remodeling of Tibial Mid-Shaft in Ovary-Intact Female Rabbits Given rhPTH 1-34 (LY333334) at 10 µg/kg (PTH10) or 40 µg/kg (PTH40) Once Daily for 5 Months<sup>a</sup>

Parameter	Abbreviations	Control	PTH10	PTH40
Resorption cavity number	RvN/CtAr (#/mm <sup>2</sup> )	0.014 ± 0.013	0.013 ± 0.004	0.097 ± 0.036 <sup>b,c</sup>
Labeled osteon number	LOn/CtAr (#/mm <sup>2</sup> )	0.011 ± 0.006	0.027 ± 0.006	0.215 ± 0.048 <sup>b,c</sup>
Osteoid thickness	OTh (µm)	4.92 ± 0.54	5.42 ± 0.30	5.16 ± 0.27
Mineral apposition rate	MAR (µm/day)	1.19 ± 0.20	1.56 ± 0.13 <sup>b</sup>	1.60 ± 0.12 <sup>b</sup>
Bone formation rate	BFR/BV (%/yr)	0.5 ± 0.3	8.5 ± 2.9 <sup>b</sup>	21.4 ± 3.8 <sup>b</sup>
Activation frequency	AcF (#/mm <sup>2</sup> -yr)	1.8 ± 1.0	15.1 ± 5.0 <sup>b</sup>	43.8 ± 10.5 <sup>b,c</sup>
Bone area	B.Ar (mm <sup>2</sup> )	29.1 ± 1.3	33.3 ± 1.9	37.8 ± 2.7 <sup>b</sup>
Marrow area	Ma.Ar (mm <sup>2</sup> )	12.7 ± 0.7	11.9 ± 1.0	10.7 ± 1.0
Cortical area	Ct.Ar (mm <sup>2</sup> )	16.4 ± 0.9	21.3 ± 1.2 <sup>b</sup>	27.1 ± 2.0 <sup>b,c</sup>
% Cortical area	%CtAr (%)	56.4 ± 1.5	64.2 ± 1.6 <sup>b</sup>	71.6 ± 1.9 <sup>b,c</sup>

<sup>a</sup> Data are expressed as mean ± SEM for 6 rabbits per group.

<sup>b</sup> p < 0.05 compared with control.

<sup>c</sup> p < 0.05 compared with PTH(1-34) 10 µg/kg/day.

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In the centrum of LV3, most formation parameters [osteoid surface (OS/BS), osteoblast surface (ObS/BS), osteoid volume (OV/TV), mineralizing surface (MS/BS), and bone formation rate (BFR/BS)] increased with PTH treatment with significant effects in the PTH40 group. Moreover, eroded surface (ES/BS) and osteoclast surface (OcS/BS) were increased in both groups. There was no effect on osteoid thickness (OTh). Fractional bone volume (BV/TV) and trabecular thickness (Tb.Th) were not changed upon 5 months of PTH treatment. Tunneling resorption and peritrabecular fibrosis were not observed in any of the groups.

### SUMMARY

In the femoral midshaft, LY333334 (10 or 40 ukd) caused an increase in X-area and BMD, and an increase in strength with an increase in cross-sectional moment of inertia (CSMI).

In the tibial midshaft, LY increased intracortical bone turnover and cortical porosity, particularly in the endocortical zone, and increased cortical bone area. In the tibial midshaft LY increased periosteal and endocortical bone formation.

In the vertebrae, LY increased cancellous bone turnover, but LY had no effect on whole tissue BMD, X-area, biomechanical properties including compressive strength, or vertebral cortical bone thickness. LY caused a slight increase in serum calcium and an increase in serum alkaline phosphatase at the high dose.

### Comments on cortical porosity, moment of inertia, and bone strength

An increase in CSMI can occur despite increased intracortical porosity if the increased porosity is compensated for by a sufficient increase in bone thickness (the latter of which was observed in the femur). Also, if the increased porosity is located relatively close to the bone's bending axis (as suggested by tibial data), it will have relatively little effect on CSMI. In fact, in a separate study the actual CSMI in the femur was re-calculated on the basis of the porosity distribution measured in the tibia, and it was shown that this actual CSMI was only slightly affected by the increase in porosity (Fig.5, from: "Changes in Geometry and Cortical Porosity in Adult, Ovary-Intact Rabbits after 5 Months Treatment with LY333334 (hPTH 1-34), by Hirano et al. (2000), *Calcif. Tissue Int.* 66, pp456-460).

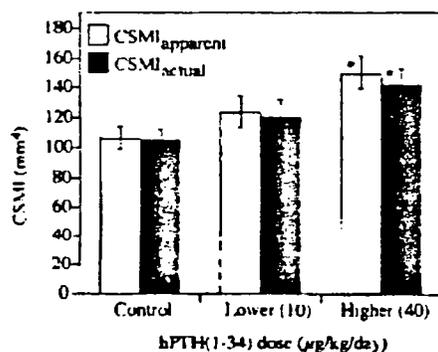


Fig. 5. Data are expressed as mean  $\pm$  SEM. \* $P < 0.05$  versus controls. Both CSMI<sub>apparent</sub> and CSMI<sub>actual</sub> in the higher dose PTH group were significantly greater than in controls. CSMI<sub>actual</sub> was smaller than CSMI<sub>apparent</sub> in the same group by 1.3% in the control group, 2.3% in the lower dose PTH group, and 4.8% in the higher dose PTH group due to porosity.

However, the main reason for the increase in midfemur strength upon treatment with PTH was the increased X-area. Since the femur was the only site tested for strength, it is not clear whether increased strength occurred only in the femur or would also be seen in the tibia or other cortical bone sites. In other words, it cannot be excluded that increased porosity with a resulting decrease in cortical BMD could lead to a decrease in (CSMI and) bone strength at some sites upon treatment with PTH. This potential adverse effect would occur if sufficient compensation by enhanced periosteal bone apposition and increased bone thickness is not achieved.

## **CONCLUSIONS**

Treatment of intact rabbits with 10 or 40  $\mu$ g LY333334 for 140 days caused an increase in femoral midshaft cortical bone strength and thickness, and an increase in tibial midshaft cortical bone turnover, surface apposition, thickness and (mainly endocortical) porosity. The results of the study suggest that the effect of PTH on cortical bone strength at any site will be determined by the balance between the effect on cortical surface bone apposition and thickness and the effect on bone turnover and porosity.

## **Effects of Human PTH (1-34), LY333334, on Bone Mass, Remodeling, and Mechanical Properties of Cortical Bone During the First Remodeling Cycle in Rabbits**

(Nonclinical Pharmacology Report CG3-13)

Based on the results of the first rabbit study, a second study was carried out in rabbits to examine the effects of PTH on the remodeling dynamics and mechanical properties of cortical bone during the first remodeling cycle (70 days) after the initiation of treatment.

The first study had shown that LY increases cortical bone mass and mechanical strength after 140 days of treatment. However, cortical porosity also increased. If cortical porosity increases prior to the change in geometry, there may be a transient decrease in cortical bone strength that could make the bone more susceptible to fracture in the early phase of treatment.

*Note: The manuscript (Mashiba et al.) describing this study was submitted to J Clin Endocrinology Metab (2000)*

## **METHODS**

Intact, 9-month-old female New Zealand white rabbits were randomized into groups of 10 animals each. A baseline control group was sacrificed at the start of the experiment. The PTH-treated groups were given rhPTH(1-34) at 10  $\mu$ g/kg daily subcutaneously for 35 (P35), or 70 (P70) days. Age-matched control groups (V35, V70) were injected with vehicle.

## **RESULTS**

There was no effect of LY (10  $\mu$ g/kg/day) on serum Ca, P, or alkaline phosphatase.

There were no sustained effects on X-area or BMD in whole femur, femoral midshaft or whole tibia. On D35 tibial BMD, and on D70 femoral midshaft BMD were slightly increased. Vertebral BMD was not measured.

In tibial midshaft there was increased cortical remodeling, as indicated by increased resorption cavity number, osteoid area, labeled osteon number, Ac.F, and BFR as compared to age-matched vehicle controls at both 35 and 70 days. Tibial midshaft strength (Fu, stiffness) was increased at 35 and 70 days. Work to failure (AUC) was (non-significantly) increased on D35 but not affected on D70.

Cortical area was increased at D35 and D70. Porosity was slightly but not significantly increased at both days. Medullary area was unaffected. Tibial periosteal bone formation was increased at D35 and D70, endocortical bone formation at D35 only. Results from the previous study (CG3-06) had shown that by 140 days the surface effects were no longer significant at the 10  $\mu$ g/kg/day dose.

These results indicate increased endocortical, periosteal and intracortical bone formation without a significant porosity increase in the first remodeling cycle, with a net result of increased bone thickness and bone strength.

In the lumbar vertebra, LY did not have a sustained effect on cancellous bone volume or bone turnover. However, it did increase Fu, stiffness, and work to failure (AUC) on both D35 and D70. In the femur midshaft, LY increased Fu, stiffness and AUC (extrinsic properties) on D35 and D70, and increased ultimate stress, modulus and toughness (intrinsic properties) on D70.

## **CONCLUSIONS**

Upon start of treatment of intact rabbits with 10  $\mu$ g/kg LY333334, cortical bone turnover and bone apposition in the tibia were increased within the first remodeling period (70 days). However, this did not lead to significantly increased porosity or an adverse effect on cortical bone strength. In fact, treatment caused an increased cortical bone thickness and increased cortical bone strength in the

tibial midshaft during the whole remodeling period. Bone strength was also increased in the femoral midshaft throughout the 70-day study period.

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## RAT BONE QUALITY STUDIES

Report	Title	In Vivo		In Vitro		Results
		Species, Strain, Gender, & Age	Dose/Route	Assay Type	Tissue/ Cell Line	
R00790 & R04296	A Study in Intact Male and Ovariectomized Female Fischer 344 Rats Given Recombinant Human Parathyroid Hormone, rhPTH 1-34 (LY333334), for 1 Year to Assess Effects on Bone	Rat, F344, intact male & OVX female, ca 6 months	8 & 40 $\mu\text{g}/\text{kg}/\text{day}$ SC once daily for 12 months			LY333334 was anabolic in skeletons of intact males & OVX female rats, increasing bone mass & biomechanical strength properties of bone in clinically relevant sites such as spine & femur neck as well as in proximal tibia & femur mid-shaft. Increases in bone mass & strength exceeded those of age-matched sham & OVX controls. In femoral mid-shaft in rats given the higher dose, ultimate strain decreased despite increase in stiffness & ultimate load strength. No abnormal histopathology was found in tibia diaphyseal-metaphyseal junctional area. Systemic growth was slightly decreased in males but not affected in OVX females. There were sex differences in femur length, but LY333334 did not alter length of tibia or femur in either sex. In treated rats, serum calcium increased by 5-10% at 6 months & then remained stable; serum alkaline phosphatase increased by 3-6%.
BNS-01	Recombinant Human Parathyroid Hormone (1-34) Effects on Bone Mass, Architecture, and Quality in Aged Ovariectomized Rats	Rat, Sprague-Dawley, female, ca 9 months, OVX	8 or 40 $\mu\text{g}/\text{kg}/\text{day}$ for 6 months SC			PTH stimulated bone formation in cancellous & cortical bone sites with improved biomechanical properties in vertebra, femoral neck, & diaphysis.
BNS-02	Skeletal Effects of LY333334 in Ovariectomized Rats with Established Osteopenia	Rat, Sprague-Dawley female, 3-6 months + 1 month post-OVX	0 to 80 $\mu\text{g}/\text{kg}/\text{day}$ for 0.5 to 6 months SC			Osteopenic rats were permitted to lose bone due to ovariectomy before initiation of dosing. PTH restored bone in the proximal tibia & distal femur to above sham & baseline levels with a minimally efficacious dose of 0.3 to 1 $\mu\text{g}/\text{kg}/\text{day}$ .
BNS-09	Skeletal Effects of LY333334 in Young and Mature Ovary-Intact Rats	Rat, F344 & Sprague-Dawley, female, 1.5 to 6 months	0-75 $\mu\text{g}/\text{kg}/\text{day}$ for 3-9 months SC			Bone mass was dose dependently increased in response to LY333334 with little change in geometry. Intact rats showed increasing responsiveness to LY333334 with maturation.
R43-01	Pharmacokinetic Profile of Human Parathyroid Hormone (1-34) (LY333334) and Serum Chemistry in Rats After Anabolic or Catabolic Injection Protocols	Rat, Sprague-Dawley, male, ca 5 weeks	80 $\mu\text{g}/\text{kg}$ once/day or 13.3 $\mu\text{g}/\text{kg}$ 6 x/day at 10 min or 1 hr intervals; SC			Anabolic or catabolic response of bone to LY333334 may be primarily determined by the length of time serum concentrations remain above baseline & only secondarily by $C_{\text{max}}$ obtained.
CG3-02	The Frequency of Daily Injections Determines Induction of an Anabolic Response in Bone by Human Synthetic and Biosynthetic (LY333334) Parathyroid Hormone Fragment, hPTH 1-34, in Young Rats	Rat, Sprague-Dawley, male, 4-6 weeks	Synthetic hPTH (1-34) or LY333334 at 80 $\mu\text{g}/\text{kg}/\text{day}$ given as 1, 3, or 6 injections within 1 hr or within a 6-8 hr period each day, or 3 injections at 80 $\mu\text{g}/\text{kg}$ over 6-hr period (daily dose 240 $\mu\text{g}/\text{kg}/\text{day}$ ) SC			PTH increased bone mass & strength when given at 80 $\mu\text{g}/\text{kg}/\text{day}$ in 1, 3, or 6 doses within a 1-hr period. If given as 80 $\mu\text{g}/\text{kg}$ 3/day (total daily dose 240 $\mu\text{g}/\text{kg}$ ), or as 6-day over 6-8 hr (total daily dose of 80 $\mu\text{g}/\text{kg}/\text{day}$ ), bone mass decreased consistent with a catabolic response. Together with companion report (R43-01) these data suggest that response in bone to PTH (1-34) may be primarily determined by length of time serum concentrations of PTH remain above baseline & only secondarily by $C_{\text{max}}$ obtained.

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## LONG TERM RAT BONE QUALITY STUDY

### A Study in Intact Male and Ovariectomized Female fischer Rats Given Recombinant Human Parathyroid Hormone, rhPTH 1-34 (LY333334) for 1 Year to Assess Effects on Bone (Study Nr. R00796/R04296)

(Non-Clinical Pharmacology Report RN00796/R04296)

#### METHODS

Studies: R00796 (1-year treatment data)  
R04296 (baseline data)

Live-phase duration: 1 year

Live-phase dates: R00496: 27 February 1996 through 27 February 1996  
R00796: 12 February 1996 through 11 February 1997

Test article: Recombinant human parathyroid hormone (1-34), LY333334 (Lilly)

Chemical name: rhPTH (1-34)

Lot number and potency: PPD03482; 1.02 mg/vial

Species and strain: Fischer 344 rats

Initial body weight range: Intact males: 323 to 383g; sham females: 167 to 197 g; OVX females: 196 to 236 g

Initial age: 12-14 weeks at time of surgery (sham or OVX)

Age at study start: 18-20 weeks (Study R00796); 20-22 weeks (Study R04296) (4-5 months)

Time of surgery to study start: 6 weeks

Route: Subcutaneous

Frequency of administration: Once daily

Number of animals: 80 males; 40 sham females; 83 OVX females (3 additional intact males and 3 OVX females were added for blood level evaluations)

Treatment groups:

Treatment groups:		Number Animals	
Group Identification	Treatment	Males	Females
Study R04296			
IntactB	Intact, vehicle control (baseline)	10	
OVXB	Ovariectomized (OVX), vehicle control (baseline)		10
ShamB	Sham-operated, vehicle control (baseline)		10
Study R00796			
IntactV	Intact, vehicle control	30	
Intact8	Intact, 8 µg LY333334/kg	20	
Intact40	Intact, 40 µg LY333334/kg	20	
OVXV	Ovariectomized, vehicle control		30
ShamV	Sham-operated, vehicle control		30
OVX8	Ovariectomized, 8 µg LY333334/kg		20
OVX40	Ovariectomized, 40 µg LY333334/kg		20

#### RESULTS

##### Body weight

LY reduced body weight gain in intact male rats. BW was increased in OVX females as compared to sham controls at study start, and remained increased throughout the study. LY had no effect on BW gain in OVX females.

### Serum calcium and alkaline phosphatase

LY333334 increased serum calcium by 5% to 10% compared to vehicle controls (male and female) after 6 months of treatment and then remained stable until the end of the study. Ovariectomy increased serum alkaline phosphatase by 30% to 50% compared to sham controls. LY333334 further increased alkaline phosphatase by 30% to 50% at 40  $\mu\text{g}/\text{kg}$  in OVX rats. A similar increase was observed for Intact40 male rats. These effects are consistent with the known actions of PTH.

### Femur length

The femurs and tibia were longer in males than females at all times in the study. LY333334 did not alter linear bone growth in males, but slightly increased the length of the long bones in OVX8 and OVX40 as compared to OVXV or ShamV.

### Bone Mass

Bone mass of proximal tibiae was analyzed by dual energy x-ray absorptiometry (DXA), while mass of femurs was analyzed by quantitative computed tomography (QCT). No data were collected for the vertebrae.

In the cancellous bone-enriched proximal tibia, LY333334 dose dependently increased bone mineral content (BMC) and bone mineral density (BMD) by up to 2- or 3-fold (at 40  $\mu\text{g}/\text{kg}$ ) in both intact males and OVX females compared to their respective controls. LY also increased projected area by up to 5-10% at 40  $\mu\text{g}/\text{kg}$  (Tables G1 and G3).

In the cortical bone of the femur mid-shaft, LY333334 dose dependently increased BMC by approximately 200% and 70%; BMD by approximately 67% and 55%; and cross-sectional area by approximately 22% and 10% in intact males and OVX females, respectively, compared to their controls (Figure 1, and Tables G2 and G4).

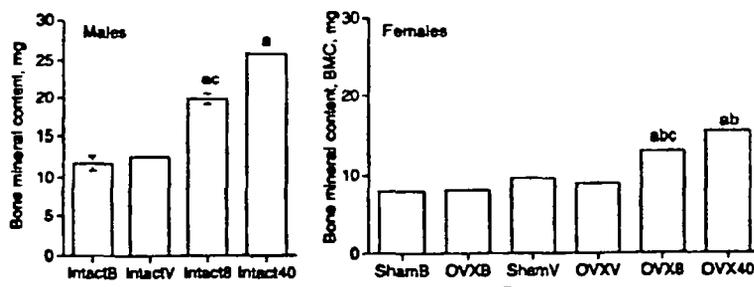


Figure 1 Bone mineral content.

Bone mineral content of the femur mid-shaft of intact male and ovariectomized female rats after treatment for 1 year with once daily LY333334 at 8 or 40  $\mu\text{g}/\text{kg}/\text{day}$ . Data are shown as mean  $\pm$  sem for 20 to 30 rats/group. Statistical significance,  $p < .05$ , a: vs controls (IntactV for males, OVXV for females); b: vs ShamV for females; c: OVX8 vs OVX40.

**Table G1** Dual Energy X-Ray Absorptiometry Analysis of Proximal Tibiae From Male Fischer 344 Rats Treated With LY333334

Group <sup>a</sup>	Length (cm)	Bone Mineral Density (g/cm <sup>2</sup> )	Bone Mineral Content (g)	Projected Area (cm <sup>2</sup> )
IntactB	1.420 ± 0.020 <sup>c,d,e</sup>	0.119 ± 0.002 <sup>d,e</sup>	0.105 ± 0.003 <sup>c,d,e</sup>	0.879 ± 0.015 <sup>c,d,e</sup>
IntactV	1.480 ± 0.010 <sup>b</sup>	0.130 ± 0.001 <sup>d,e</sup>	0.126 ± 0.001 <sup>b,d,e</sup>	0.964 ± 0.011 <sup>b</sup>
Intact8	1.495 ± 0.014 <sup>b</sup>	0.236 ± 0.002 <sup>b,c,e</sup>	0.231 ± 0.003 <sup>b,c,e</sup>	0.981 ± 0.009 <sup>b</sup>
Intact40	1.472 ± 0.016 <sup>b</sup>	0.326 ± 0.003 <sup>b,c,d</sup>	0.320 ± 0.006 <sup>b,c,d</sup>	0.978 ± 0.011 <sup>b</sup>

- <sup>a</sup> Mean ± SEM.
- <sup>b</sup> Significantly different from IntactB, p<.05.
- <sup>c</sup> Significantly different from IntactV, p<.05.
- <sup>d</sup> Significantly different from Intact8, p<.05.
- <sup>e</sup> Significantly different from Intact40, p<.05.

**Table G2** Quantitative Computed Tomography Analysis of Femoral Mid-Shaft From Male Fischer 344 Rats Treated With LY333334

Group <sup>a</sup>	Bone Mineral Density (mg/cc)	Bone Mineral Content (g)	X-Area (mm <sup>2</sup> )	Voxel Number
IntactB	890 ± 31 <sup>c,d,e</sup>	11.8 ± 0.90 <sup>d,e</sup>	10.9 ± 0.37 <sup>c,d,e</sup>	501 ± 17 <sup>c,d,e</sup>
IntactV	796 ± 9 <sup>b,d,e</sup>	12.6 ± 0.18 <sup>d,e</sup>	13.3 ± 0.19 <sup>b,d,e</sup>	609 ± 9 <sup>b,d,e</sup>
Intact8	1161 ± 6 <sup>b,c,e</sup>	19.8 ± 0.71 <sup>b,c,e</sup>	14.2 ± 0.50 <sup>b,c,e</sup>	678 ± 8 <sup>b,c,e</sup>
Intact40	1332 ± 7 <sup>b,c,d</sup>	25.8 ± 0.31 <sup>b,c,d</sup>	16.2 ± 0.18 <sup>b,c,d</sup>	742 ± 8 <sup>b,c,d</sup>

- <sup>a</sup> Mean ± SEM.
- <sup>b</sup> Significantly different from IntactB, p<.05.
- <sup>c</sup> Significantly different from IntactV, p<.05.
- <sup>d</sup> Significantly different from Intact8, p<.05.
- <sup>e</sup> Significantly different from Intact40, p<.05.

**Table G3** Dual Energy X-Ray Absorptiometry Analysis of Proximal Tibiae From Female Fischer 344 Rats Treated With LY333334

Group <sup>a</sup>	Length (cm)	Bone Mineral Density (g/cm <sup>2</sup> )	Bone Mineral Content (g)	Projected Area (cm <sup>2</sup> )
OVXE	1.300 ± 0.045	0.099 ± 0.001 <sup>c,d,e</sup>	0.073 ± 0.002 <sup>b,c,d,e</sup>	0.736 ± 0.026
ShamE	1.250 ± 0.027 <sup>b,d,e</sup>	0.112 ± 0.002 <sup>c,d,e</sup>	0.078 ± 0.001 <sup>c,d,e</sup>	0.698 ± 0.018 <sup>b,c,d,e</sup>
OVXV	1.300 ± 0.011	0.110 ± 0.001 <sup>c,d,e</sup>	0.082 ± 0.001 <sup>c,d,e</sup>	0.749 ± 0.007
ShamV	1.300 ± 0.005	0.140 ± 0.007 <sup>b,d,e</sup>	0.103 ± 0.002 <sup>b,d,e</sup>	0.770 ± 0.006
OVX8	1.333 ± 0.011 <sup>b,c</sup>	0.176 ± 0.002 <sup>b,c,e</sup>	0.137 ± 0.002 <sup>b,c,e</sup>	0.779 ± 0.007 <sup>b</sup>
OVX40	1.330 ± 0.011 <sup>b,c</sup>	0.224 ± 0.003 <sup>b,c,d</sup>	0.176 ± 0.002 <sup>b,c,d</sup>	0.787 ± 0.008 <sup>b</sup>

- <sup>a</sup> Mean ± SEM.
- <sup>b</sup> Significantly different from OVXV, p<.05.
- <sup>c</sup> Significantly different from ShamV, p<.05.
- <sup>d</sup> Significantly different from OVX8, p<.05.
- <sup>e</sup> Significantly different from OVX40, p<.05.

**Table G4** Quantitative Computed Tomography Analysis of Femoral Mid-Shaft From Female Fischer 344 Rats Treated With LY333334

Group <sup>a</sup>	Bone Mineral Density (mg/cc)	Bone Mineral Content (g)	X-Area (mm <sup>2</sup> )	Voxel Number
OVXE	836 ± 9 <sup>c,d,e</sup>	7.98 ± 0.14 <sup>c,d,e</sup>	7.96 ± 0.13 <sup>b,c</sup>	366 ± 5.8 <sup>b,c</sup>
ShamE	860 ± 9 <sup>b,c,d,e</sup>	7.89 ± 0.17 <sup>c,d,e</sup>	7.65 ± 0.15 <sup>b,c</sup>	351 ± 6.7 <sup>b,c</sup>
OVXV	812 ± 7 <sup>c,d,e</sup>	8.92 ± 0.11 <sup>c,d,e</sup>	9.15 ± 0.08	420 ± 8.6
ShamV	904 ± 18 <sup>b,d,e</sup>	9.64 ± 0.30 <sup>b,d,e</sup>	8.88 ± 0.19	408 ± 8.6
OVX8	1105 ± 10 <sup>b,c,e</sup>	12.9 ± 0.18 <sup>b,c,e</sup>	9.74 ± 0.09 <sup>b,c</sup>	447 ± 4.2 <sup>b,c</sup>
OVX40	1258 ± 20 <sup>b,c,d</sup>	15.3 ± 0.35 <sup>b,c,d</sup>	10.1 ± 0.13 <sup>b,c</sup>	464 ± 6.0 <sup>b,c</sup>

- <sup>a</sup> Mean ± SEM.
- <sup>b</sup> Significantly different from OVXV, p<.05.
- <sup>c</sup> Significantly different from ShamV, p<.05.
- <sup>d</sup> Significantly different from OVX8, p<.05.
- <sup>e</sup> Significantly different from OVX40, p<.05.

In summary, LY333334 increased bone mass (BMC and BMD) and bone geometry in a dose-dependent manner in both cancellous and cortical bone regions of intact male and ovariectomized female Fischer 344 rats.

### **Bone Strength**

Bone strength was determined in lumbar vertebrae (compression), femur midshaft (3-point bending), and femur neck (load to failure). X-area, BMD and BMC of femoral midshaft were determined by pQCT.

#### Biomechanical parameters (Figure H1)

$F_u$	ultimate load
$d_u$	ultimate displacement (1/brittleness)
$S$	stiffness
$U$	work to failure
$S_u$	ultimate stress (inversely proportional to $I$ )
$I$	moment of inertia (increases with $t$ )
$E$	Young's modulus
$u$	modulus of toughness
$\epsilon_u$	ultimate strain (in vertebra: $d_u/l$ )
$t$	cortical thickness or, in vertebra: specimen thickness

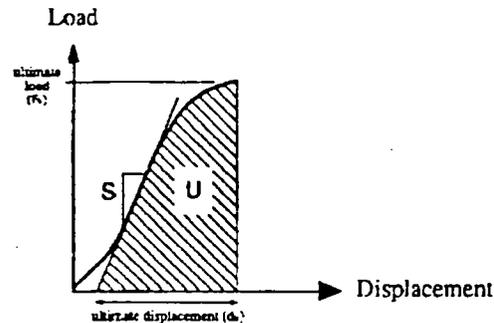


Figure H1 Derivation of biomechanical parameters from the load/displacement curves.

Note: Ultimate load ( $F_u$ ), which reflects the extrinsic bone strength, is the maximum height of the curve. Ultimate displacement ( $d_u$ ), which is the reciprocal of bone brittleness, is the maximum width of the curve. Stiffness ( $S$ ) is the maximum slope and work to failure ( $U$ ) is the area under the curve.

### **Data for vertebrae and femur**

#### **Biomechanical strength**

1. In lumbar vertebra L-6 LY333334 increased strength by approximately <200% in males (Figure 2) and females (Figure 3).
2. In the femur neck, LY333334 increased strength by 150% to 175% in males (Figure 2) and by 140% to 175% in females compared to OVXV or by 120% to 150% compared to ShamV (Figure 3).
3. In the femur mid-shaft, LY333334 increased strength by  $\geq 250\%$  in both males and females (Figure 2,3).

#### **Other biomechanical parameters**

Ultimate strain decreased slightly at the higher dose (Intact40 and OVX40), while stiffness, Young's modulus, ultimate load, and ultimate stress (strength) increased for both males and females (Tables H1-H2).

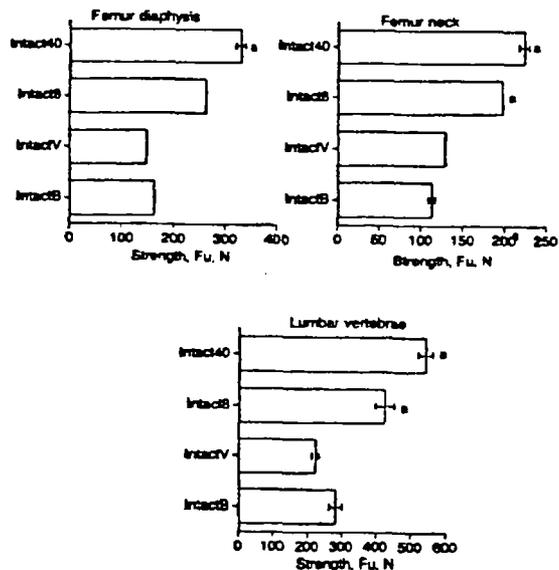


Figure 2 Biomechanical strength - males.

Biomechanical strength, measured as ultimate load (Fu) of the femur diaphyseal mid-shaft (cortical bone site), femur neck (cortical and trabecular bone), and lumbar vertebrae (trabecular bone-enriched site) in male rats, after treatment for 1 year with once daily LY333334 at 8 or 40  $\mu\text{g}/\text{kg}/\text{day}$ . Data are shown as mean  $\pm$  sem for 20 to 30 rats/group. Statistical significance,  $p < .05$ , a: vs IntactV controls.

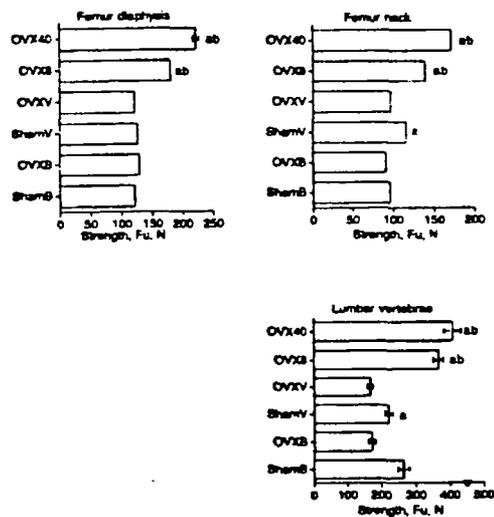


Figure 3 Biomechanical strength - females.

Biomechanical strength, measured as ultimate load (Fu) of the femur diaphyseal mid-shaft (cortical bone site), femur neck (cortical and trabecular bone), and lumbar vertebrae (trabecular bone-enriched site) in ovariectomized female rats after treatment for 1 year with once daily LY333334 at 8 or 40  $\mu\text{g}/\text{kg}/\text{day}$ . Data are shown as mean  $\pm$  sem for 20 to 30 rats/group. Statistical significance,  $p < .05$ , a: vs OVXV; b: vs ShamV; c: OVX8 vs OVX40.

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**Table H1 Biomechanical Parameters for Male Rats Treated With LY333334**

Variable	IntactB	IntactV	Intact8	Intact40
<b>Femur Diaphyseal Midshaft<sup>a</sup></b>				
F <sub>u</sub> (N)	162 ± 3	147 ± 2	262 ± 4 <sup>bc</sup>	329 ± 9 <sup>bc</sup>
S (N/mm)	413 ± 12	363 ± 9 <sup>b</sup>	653 ± 10 <sup>bc</sup>	806 ± 20 <sup>bc</sup>
U (mJ)	72 ± 2	63 ± 2	84 ± 4 <sup>bc</sup>	78 ± 4 <sup>c</sup>
d <sub>1/2</sub> (mm)	0.68 ± 0.02	0.68 ± 0.02	0.53 ± 0.02 <sup>bc</sup>	0.44 ± 0.01 <sup>bc</sup>
S <sub>y</sub> (Mpa)	239 ± 6	220 ± 2 <sup>b</sup>	272 ± 6 <sup>bc</sup>	277 ± 9 <sup>bc</sup>
E (GPa)	7.8 ± 0.2	10.9 ± 0.3 <sup>b</sup>	13.1 ± 0.6 <sup>bc</sup>	11.9 ± 0.4 <sup>b</sup>
u (MJ/m <sup>3</sup> )	8.3 ± 0.3	4.7 ± 0.1 <sup>b</sup>	4.6 ± 0.2 <sup>c</sup>	3.8 ± 0.2 <sup>bc</sup>
ε <sub>u</sub>	0.053 ± 0.002	0.034 ± 0.001 <sup>b</sup>	0.028 ± 0.001 <sup>bc</sup>	0.025 ± 0.001 <sup>bc</sup>
<b>Femoral Neck<sup>a</sup></b>				
F <sub>u</sub> (N)	113 ± 4	129 ± 2 <sup>b</sup>	197 ± 3 <sup>bc</sup>	223 ± 6 <sup>bc</sup>
S (N/mm)	115 ± 8	406 ± 34 <sup>b</sup>	449 ± 30 <sup>b</sup>	499 ± 18 <sup>bc</sup>
U (mJ)	63 ± 5	42 ± 3 <sup>b</sup>	62 ± 6 <sup>c</sup>	58 ± 4 <sup>c</sup>
d <sub>1/2</sub> (mm)	1.08 ± 0.07	0.55 ± 0.03 <sup>a</sup>	0.58 ± 0.05 <sup>b</sup>	0.49 ± 0.03 <sup>a</sup>
<b>Lumbar Vertebra<sup>a</sup></b>				
F <sub>u</sub> (N)	282 ± 19	222 ± 10	424 ± 27 <sup>bc</sup>	541 ± 21 <sup>bc</sup>
S (N/mm)	2412 ± 258	1623 ± 93 <sup>b</sup>	2605 ± 239 <sup>c</sup>	2972 ± 218 <sup>c</sup>
U (mJ)	25.6 ± 1.1	24.1 ± 1.5	63.5 ± 6.6 <sup>bc</sup>	81.9 ± 6.7 <sup>bc</sup>
d <sub>1/2</sub> (mm)	0.16 ± 0.01	0.19 ± 0.01	0.26 ± 0.03 <sup>bc</sup>	0.25 ± 0.02 <sup>bc</sup>
S <sub>y</sub> (Mpa)	32.5 ± 1.0	22.0 ± 0.8 <sup>b</sup>	40.9 ± 2.1 <sup>bc</sup>	43.9 ± 1.5 <sup>bc</sup>
E (MPa)	726 ± 67	582 ± 37	941 ± 90 <sup>c</sup>	882 ± 67 <sup>c</sup>
u (MJ/m <sup>3</sup> )	1.17 ± 0.06	0.67 ± 0.03 <sup>b</sup>	1.63 ± 0.14 <sup>bc</sup>	1.77 ± 0.08 <sup>bc</sup>
ε <sub>u</sub>	0.059 ± 0.004	0.053 ± 0.003	0.069 ± 0.007 <sup>c</sup>	0.070 ± 0.004 <sup>c</sup>

<sup>a</sup> Mean ± sem.

<sup>b</sup> Significantly different than IntactB.

<sup>c</sup> Significantly different than IntactV.

**Table H2 Biomechanical Parameters For Female Rats Treated With LY333334**

Variable	ShamB	OVXB	ShamV	OVXV	OVX8	OVX40
<b>Femur Diaphyseal Midshaft<sup>a</sup></b>						
F <sub>u</sub> (N)	121 ± 3	129 ± 2	127 ± 2	121 ± 2	180 ± 3 <sup>bcde</sup>	222 ± 5 <sup>bcde</sup>
S (N/mm)	342 ± 7	351 ± 9	435 ± 10 <sup>bc</sup>	402 ± 6 <sup>bcde</sup>	618 ± 13 <sup>bcde</sup>	783 ± 12 <sup>bcde</sup>
U (mJ)	45 ± 3	60 ± 4 <sup>b</sup>	32 ± 1 <sup>bc</sup>	35 ± 2 <sup>bc</sup>	44 ± 3 <sup>cd</sup>	39 ± 2 <sup>cd</sup>
d <sub>1/2</sub> (mm)	0.58 ± 0.02	0.69 ± 0.03 <sup>b</sup>	0.41 ± 0.01 <sup>bc</sup>	0.46 ± 0.02 <sup>bcde</sup>	0.41 ± 0.02 <sup>bcde</sup>	0.32 ± 0.01 <sup>bcde</sup>
S <sub>y</sub> (Mpa)	260 ± 4	258 ± 6	236 ± 3 <sup>bc</sup>	212 ± 3 <sup>bcde</sup>	255 ± 4 <sup>d</sup>	272 ± 6 <sup>d</sup>
E (GPa)	10.3 ± 0.1	9.6 ± 0.3	10.7 ± 0.2 <sup>c</sup>	9.2 ± 0.2 <sup>bd</sup>	11.0 ± 0.2 <sup>c</sup>	11.6 ± 0.2 <sup>bcde</sup>
u (MJ/m <sup>3</sup> )	7.0 ± 0.5	8.8 ± 0.6 <sup>b</sup>	4.4 ± 0.1 <sup>bc</sup>	4.7 ± 0.2 <sup>bc</sup>	5.0 ± 0.3 <sup>bc</sup>	3.9 ± 0.2 <sup>bc</sup>
ε <sub>u</sub>	0.041 ± 0.002	0.050 ± 0.002 <sup>b</sup>	0.031 ± 0.001 <sup>bc</sup>	0.035 ± 0.001 <sup>bcde</sup>	0.032 ± 0.001 <sup>bc</sup>	0.026 ± 0.001 <sup>bcde</sup>
<b>Femoral Neck<sup>a</sup></b>						
F <sub>u</sub> (N)	96 ± 3	91 ± 3	116 ± 2 <sup>bc</sup>	97 ± 3 <sup>d</sup>	139 ± 3 <sup>bcde</sup>	171 ± 3 <sup>bcde</sup>
S (N/mm)	130 ± 10	129 ± 9	296 ± 16 <sup>bc</sup>	256 ± 16 <sup>bc</sup>	369 ± 42 <sup>bcde</sup>	391 ± 20 <sup>bcde</sup>
U (mJ)	41 ± 3	39 ± 3	32 ± 2 <sup>b</sup>	23 ± 1 <sup>bcde</sup>	40 ± 3 <sup>d</sup>	46 ± 3 <sup>d</sup>
d <sub>1/2</sub> (mm)	0.83 ± 0.05	0.82 ± 0.06	0.50 ± 0.03 <sup>bc</sup>	0.45 ± 0.02 <sup>bc</sup>	0.53 ± 0.04 <sup>bc</sup>	0.51 ± 0.03 <sup>bc</sup>
<b>Lumbar Vertebra<sup>a</sup></b>						
F <sub>u</sub> (N)	265 ± 16	172 ± 9 <sup>b</sup>	222 ± 11 <sup>c</sup>	167 ± 8 <sup>bd</sup>	366 ± 14 <sup>bcde</sup>	408 ± 25 <sup>bcde</sup>
S (N/mm)	2253 ± 287	1253 ± 179 <sup>b</sup>	1780 ± 118 <sup>c</sup>	1485 ± 88 <sup>b</sup>	2755 ± 168 <sup>bcde</sup>	2443 ± 195 <sup>bcde</sup>
U (mJ)	27.0 ± 2.1	23.5 ± 3.9	23.7 ± 2.1	17.6 ± 1.5 <sup>b</sup>	40.4 ± 3.5 <sup>bcde</sup>	55.1 ± 4.3 <sup>bcde</sup>
d <sub>1/2</sub> (mm)	0.17 ± 0.02	0.22 ± 0.04	0.18 ± 0.01	0.17 ± 0.01 <sup>c</sup>	0.18 ± 0.01 <sup>c</sup>	0.23 ± 0.01 <sup>bcde</sup>
S <sub>y</sub> (Mpa)	40.2 ± 1.7	27.9 ± 1.3 <sup>b</sup>	30.3 ± 1.2 <sup>b</sup>	21.7 ± 0.8 <sup>bcde</sup>	45.0 ± 1.1 <sup>bcde</sup>	46.7 ± 1.5 <sup>bcde</sup>
E (MPa)	737 ± 72	518 ± 81 <sup>b</sup>	687 ± 46	546 ± 32 <sup>bd</sup>	1004 ± 64 <sup>bcde</sup>	810 ± 57 <sup>c</sup>
u (MJ/m <sup>3</sup> )	1.86 ± 0.13	1.59 ± 0.26	1.14 ± 0.09 <sup>bc</sup>	0.82 ± 0.07 <sup>bcde</sup>	1.67 ± 0.11 <sup>d</sup>	2.19 ± 0.15 <sup>bcde</sup>
ε <sub>u</sub>	0.076 ± 0.006	0.097 ± 0.019	0.063 ± 0.004 <sup>c</sup>	0.060 ± 0.004 <sup>c</sup>	0.063 ± 0.004 <sup>c</sup>	0.081 ± 0.006 <sup>bcde</sup>

<sup>a</sup> Mean ± sem.

<sup>b</sup> Significantly different than ShamB.

<sup>c</sup> Significantly different than OVXB.

<sup>d</sup> Significantly different than ShamV.

<sup>e</sup> Significantly different than OVXV.

Data on correlation between BMC and Fu (femur midshaft)

Morphometry and BMC/BMD data (QCT) for the femur midshaft are shown in Tables H3-H4. The moment of inertia of the femoral midshaft increased by 47% in OVX40 females and by 104% in Intact40 males. This increase was related to the increase in femoral thickness (t). These findings indicate that the agent substantially increased periosteal bone formation.

Figure H2 shows the relation between femoral midshaft ultimate load and BMC. This suggests that bone strength was significantly correlated with total bone mineral, which is proportional to BMD and bone area (or size).

**Table H3** Bone Morphometry and Mineral Content for Male Rats Treated with LY333334

Variable	IntactB	IntactV	IntactR	Intact40
		Femur*		
t (mm)	0.60 ± 0.01	0.54 ± 0.01	1.04 ± 0.01 <sup>bc</sup>	1.46 ± 0.06 <sup>bc</sup>
I (mm <sup>4</sup> )	3.74 ± 0.08	5.59 ± 0.11 <sup>b</sup>	8.53 ± 0.33 <sup>bc</sup>	11.45 ± 0.42 <sup>bc</sup>
Cortical area (mm <sup>2</sup> )	6.78 ± 0.11	8.63 ± 0.08 <sup>b</sup>	10.36 ± 0.15 <sup>bc</sup>	12.10 ± 0.24 <sup>bc</sup>
Total area (mm <sup>2</sup> )	8.13 ± 0.11	10.39 ± 0.09 <sup>b</sup>	11.46 ± 0.17 <sup>bc</sup>	12.79 ± 0.18 <sup>bc</sup>
BMC (mg)	10.9 ± 0.2	12.8 ± 0.1 <sup>b</sup>	20.6 ± 0.3 <sup>bc</sup>	25.8 ± 0.3 <sup>bc</sup>
BMD (mg/cc)	860 ± 11	791 ± 8 <sup>b</sup>	1161 ± 6 <sup>bc</sup>	1332 ± 7 <sup>bc</sup>

\* Mean ± sem.

<sup>b</sup> Significantly different than IntactB.

<sup>c</sup> Significantly different than IntactV.

**Table H4** Bone Morphometry and Mineral Content for Female Rats Treated with LY333334

Variable	ShamB	OVXB	ShamV	OVXV	OVXR	OVX40
			Femur*			
t (mm)	0.52 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.52 ± 0.06	0.75 ± 0.01 <sup>bcde</sup>	1.10 ± 0.02 <sup>bcde</sup>
I (mm <sup>4</sup> )	2.35 ± 0.07	2.59 ± 0.12	2.87 ± 0.10 <sup>bc</sup>	3.10 ± 0.10 <sup>bc,d</sup>	3.97 ± 0.06 <sup>bcde</sup>	4.57 ± 0.08 <sup>bcde</sup>
Cortical area (mm <sup>2</sup> )	3.73 ± 0.08	3.94 ± 0.10	4.14 ± 0.06 <sup>b</sup>	4.11 ± 0.07 <sup>b</sup>	5.56 ± 0.09 <sup>bcde</sup>	7.08 ± 0.11 <sup>bcde</sup>
Total area (mm <sup>2</sup> )	6.12 ± 0.13	6.45 ± 0.13	6.97 ± 0.11 <sup>bc</sup>	7.21 ± 0.07 <sup>bc</sup>	7.59 ± 0.10 <sup>bcde</sup>	7.81 ± 0.14 <sup>bcde</sup>
BMC (mg)	7.89 ± 0.17	7.98 ± 0.14	9.30 ± 0.13 <sup>bc</sup>	8.92 ± 0.11 <sup>bc,d</sup>	12.92 ± 0.18 <sup>bcde</sup>	15.60 ± 0.21 <sup>bcde</sup>
BMD (mg/cc)	860 ± 9	836 ± 9	892 ± 7 <sup>bc</sup>	812 ± 7 <sup>bc</sup>	1105 ± 16 <sup>bcde</sup>	1281 ± 8 <sup>bcde</sup>

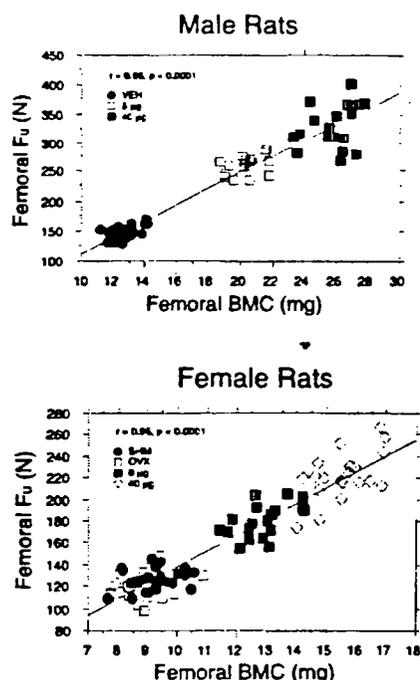
\* Mean ± sem.

<sup>b</sup> Significantly different than ShamB.

<sup>c</sup> Significantly different than OVXB.

<sup>d</sup> Significantly different than ShamV.

<sup>e</sup> Significantly different than OVXV.



**Figure H2** Correlation between ultimate load of the femur ( $F_u$ ) and the diaphyseal bone mineral content (BMC).

Note: Data from baseline rats (early controls) were not included in the above figures, thus the comparisons were limited to same age and size rats.

Biomechanical characteristics of different bone sites

Similar responses to LY333334 on bone strength were seen in females and males, however, the effect of LY333334 was site specific. Figures H3,4,5 graphically show the different biomechanical characteristics of rat vertebrae (H3), femoral neck (H4), and femoral midshaft (H5).

In lumbar vertebrae, LY333334 increased both ultimate load and ultimate displacement causing a large increase in work to failure and a reduction in brittleness (Figure H3). In the femoral neck, LY333334 increased ultimate load without changing ultimate displacement, thus work to failure was increased but there was no change in brittleness (Figure H4). In the femur, LY333334 increased ultimate load while decreasing ultimate displacement. The bones became more brittle with treatment and work to failure was only marginally improved (Figure H5). The latter result was unexpected.

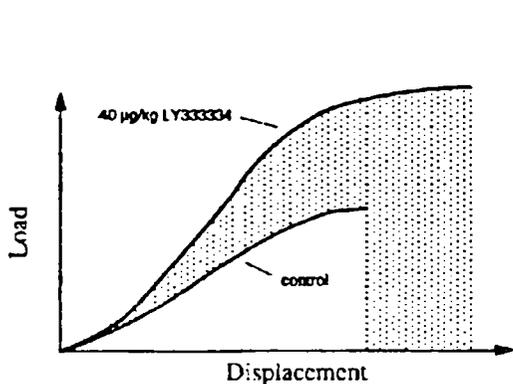


Figure H3 Effects of LY333334 on rat vertebrae.

Note: Shaded area indicates the gain in work to failure.

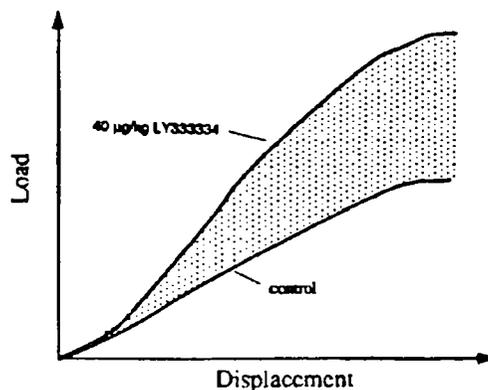


Figure H4 Effects of LY333334 on rat femoral necks.

Note: Shaded area indicates the gain in work to failure

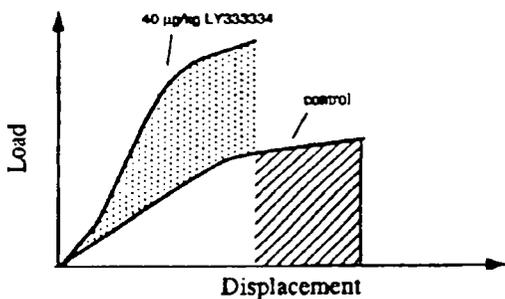


Figure H5 Effects of LY333334 on rat femoral bending test.

Note: Shaded area indicates the gain in work to failure and the striped area represents the loss in work to failure.

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In summary, LY333334 significantly and dose-dependently increased bone strength of the femur, the femoral neck and lumbar vertebra. LY333334 also changed the biomechanical characteristics of the three different bone sites in a different manner.

### Histomorphometry

Data shown are for the proximal tibia of baseline rats only, which were killed approximately 8 weeks after surgery (Study R04296).

- Trabecular bone volume (BV/TV, Tb.N) in OVX females and intact males was statistically significantly lower than in sham females. This confirmed that OVX rats were osteopenic at the initiation of dosing.
- Bone turnover (BFR), i.e, bone formation and bone resorption measures, were significantly higher in OVX females and male rats than in sham females.
- Osteoid volume in male rats was approximately 10% (0.1x) that measured in females. The reason for this was unclear as there were no equivalent differences between sexes in bone formation measures.
- Because of an error in specimen trimming and processing, data were not collected from specimens at the end of the study.

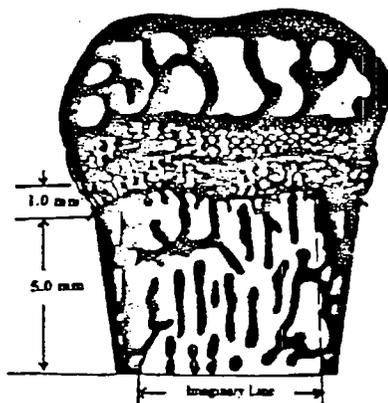


Figure 11 Schematic diagram of longitudinal section cut through upper end of tibia of a young rat. The picture illustrates measured areas between 1 and 5 mm from growth plate towards the midshaft and between the imaginary lines.

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Table 11 Histomorphometry of Proximal Tibia of Untreated Sham and Ovariectomized (OVX) Females, 8 Weeks after Surgery, and Intact Male Rats at Study Start

Measurement	Units	Baseline Sham <sup>a</sup>	Baseline OVX <sup>a</sup>	Baseline Males <sup>c</sup>
Bone volume (BV/TV)	%	14.6 ± 0.8	5.2 ± 0.5 <sup>b</sup>	8.8 ± 1.1 <sup>c</sup>
Trabecular thickness (TbTh)	μ	47.5 ± 1.0	45.5 ± 1.4	50.3 ± 1.8
Trabecular number (TbN)		3.1 ± 0.2	1.1 ± 0.1 <sup>b</sup>	1.7 ± 0.2 <sup>c</sup>
Trabecular space (TbSp)	μ	285 ± 17	911 ± 17 <sup>b</sup>	602 ± 80 <sup>c</sup>
Osteoid volume (OV/BV)	%	3.1 ± 0.2	4.3 ± 0.2	0.3 ± 0.0
Osteoid surface (OS)	%	25.9 ± 1.5	33.3 ± 1.5	29.8 ± 1.2
Osteoid thickness (OTH)	μ	3.6 ± 0.1	3.7 ± 0.1	3.8 ± 0.1
Osteoblast + surfaces (ObS/BS)	%	6.8 ± 0.8	16.2 ± 2.0 <sup>b</sup>	11.6 ± 1.7 <sup>c</sup>
Mineralizing surfaces (MS/BS)	%	25.9 ± 1.5	33.3 ± 1.5 <sup>b</sup>	29.8 ± 1.2
Mineralization rate (MAR)	μ/day	1.11 ± 0.04	1.45 ± 0.03 <sup>b</sup>	1.38 ± 0.04 <sup>c</sup>
Bone formation rate (BFR/BS)	μ/day	83 ± 6	137 ± 6 <sup>b</sup>	118 ± 6 <sup>c</sup>
Bone formation rate (BFR/BV)	%/year	351 ± 26	575 ± 38 <sup>b</sup>	473 ± 27 <sup>c</sup>
Bone formation rate (BFR/TV)	%/year	50.51 ± 4.0	30.7 ± 2.4 <sup>b</sup>	39.8 ± 4.2 <sup>c</sup>
Formation period (FP)	days	11.2 ± 0.6	13.5 ± 0.5 <sup>b</sup>	11.2 ± 0.6 <sup>c</sup>
Mineralization time (OMT)	days	3.3 ± 0.1	2.6 ± 0.1	2.8 ± 0.1
Resorption surface (ReS/BS)	%	8.5 ± 1.3	13.2 ± 1.6	12.5 ± 1.2
Eroded surface (ES/BS)	%	8.9 ± 1.3	14.4 ± 1.7 <sup>b</sup>	13.0 ± 1.3
Osteoclast + surface (OcS/BS)	%	0.32 ± 0.05	1.24 ± 0.23 <sup>b</sup>	0.51 ± 0.07 <sup>c</sup>
Quiescent surface (QS/BS)	%	65.3 ± 2.5	52.3 ± 2.3 <sup>b</sup>	57.2 ± 1.6 <sup>c</sup>

<sup>a</sup> 10 group.

<sup>b</sup> Statistical significance, p<.05, sham female versus OVX female.

<sup>c</sup> Statistical significance, p<.05, intact male versus sham female.

### Toxicokinetics

Blood samples were collected predose and 15 min postdose (near  $T_{max}$ ), after 1, 151, 334 days (Day 0, 150, 333) of dosing, from 3 animals/sex/treatment group per time point. LY was assayed  
 Quantitation limit was

Serum levels of LY333334 (ng/ml) in F344 rats treated with 8 or 40 ug/kg

Day	8 ug/kg		Human $C_{max}$ multiple*	Human AUC multiple**	40 ug/kg		Human $C_{max}$ multiple*	Human AUC multiple**
	Predose	15 min			predose	15 min		
0	NR	NR	-	-	0 (BQL)	10.93	69x	31x
150	0 (BQL)	3.18	20x	9x	0 (BQL)	13.47	85x	38x
333	0 (BQL)	3.29	21x	9.5x	0 (BQL)	14.71	93x	42x

NR= not reported, insufficient sample volume

BQL = below quantitation limit

\* Human  $C_{max}$  (15 min) = 159 pg/ml, at 20 ug daily dose

\*\* Extrapolated from 2-year rat carcinogenicity study AUC and  $C_{max}$  data (Human AUC = 295 pgxh/ml, at 20 ug daily dose)

The predose serum concentrations of LY333334 after 6 and 12 months of treatment were below the quantitation limit. This suggested there was no accumulation of LY333334 upon repeated dosing during the study.

The serum concentrations of immunoreactive LY333334 at 15 min (0.25h) postdose, were dose dependent. No differences were observed between serum levels between males and females or between Days 0, 150, 333.

### Morphologic Pathology

Kidneys from terminally necropsied animals treated with 0 or 40 ug/kg (males: IntactV or Intact40, females: OVXV or OVX40, n=19 or 20/group) were weighted and examined histologically.

All intact males and several females had progressive glomerulonephrosis (PGN). In the OVX40 group, the incidence of PGN was increased as compared to OVXV, from 10/20 to 19/20. No other effects of LY333334 on kidney histology were noted.

### CONCLUSIONS

In conclusion, LY333334 significantly increased bone mass and bone strength in intact male and OVX female rats after 1 year of once daily treatment.

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## OTHER RAT BONE STUDIES

### Recombinant Human Parathyroid Hormone (1-34) Effects on Bone Mass, Architecture, and Quality in Aged Ovariectomized Rats

(Nonclinical Pharmacology Report BN5-01)

Note: Preliminary results of this study were published by Sato et al. (*Endocrinology*, Vol. 138, #10, p4330, 1997)

This study was carried out to evaluate the effect of LY in older ovariectomized rats, and was powered adequately for biomechanical analysis.

## METHODS

Study was carried out in "aged" 9-month old SD rats (N=26 baseline group, N=35/treatment group). Groups included baseline animals, sham controls, OVX controls, OVX given 8 ug/kg LY, or OVX given 40 ug/kg LY, once daily by s.c. injection. Treatment duration was 6 months. Bone mass measurements were carried out by QCT (during in-life phase of study and at study termination), and histomorphometry and biomechanical analyses were carried out of bones removed at the end of the study.

## RESULTS

### Bone mass

In life QCT analysis of proximal tibial metaphysis showed a small (ca. 5%) age-dependent reduction in bone BMD. Ovariectomy caused a larger reduction in tibial BMD (15%). LY dose-dependently increased tibial BMD up to 2x the OVX control value, at 40 ug/kg, after 5 months.

In the femoral metaphysis (distal femur) and diaphysis, OVX significantly decreased BMD, and LY increased BMD above base-line levels at both doses (2-3x OVX control values) (Figure 3).

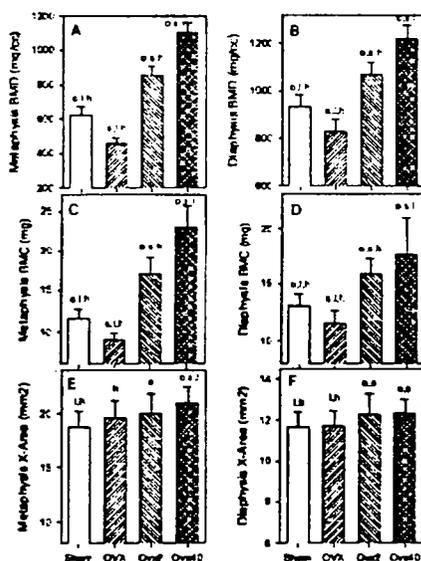


Figure 3

Analysis of femora by QCT. The metaphysis and mid-diaphysis of femora were examined at termination by QCT, using voxel dimensions of 0.147 x 0.147 x 1.2 mm. Data are mean  $\pm$  standard deviation. Significant differences from Sham, OVX, OVX8 and OVX40 are designated "s", "o", "l", and "h", respectively (Fisher PLSD,  $p < 0.011$ ). Analysis of the

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## Histomorphometry

### Cortical bone

In both tibial and femoral diaphyses cortical thickness or area were slightly reduced by OVX. LY markedly increased cortical thickness, up to 1.8x at 40 ug/kg (femur), and decreased medullary area, concomitant with an increase in moment of inertia. As shown by dynamic histomorphometry, bone formation rate at the periosteal and endocortical surface of the tibial midshaft was increased by OVX, and further increased markedly by LY in a dose-dependent manner (2-3x).

### Cancellous bone

Histomorphometric analysis showed that in the metaphyses of the tibia OVX caused a marked reduction in BV/TV and Tb.N but not Tb.Th. LY largely improved all trabecular bone parameters (BV/TV, Tb.N, Tb.Sp, Tb.Th) so that LY (40 ug/kg)-treated tibial trabeculae were much thicker and closer together than control (sham) trabeculae. LY also dose-dependently increased trabecular connectivity. Another effect of LY was an increase in bone formation (MS/BS, BFR/TV) parameters indicating a stimulation of osteoblastic activity. Histomorphometric analysis of cancellous vertebral bone showed similar effects on trabecular bone architecture as in tibial metaphysis.

## Bone strength

### Femoral sites

Biomechanical analysis of femoral diaphysis and femoral neck showed decreased bone strength in OVX animals, including a decrease in Young's modulus (E) (slope of the deformation-load curve). LY dose-dependently improved structural ("extrinsic") properties ( $F_u$ , S) and material ("intrinsic") properties ( $\sigma_u$ , E, U) above OVX and sham values. Only  $\epsilon_u$  was unaffected or minimally decreased by LY (Table 7). This was in accordance with data from the 1-year study in which this parameter (ultimate strain) in the femoral diaphysis was decreased after 1 year treatment of initially 4- to 5-mo old rats. Femoral neck strength was unaffected by OVX, but was increased by LY.

**Table 7** Biomechanical Analyses of the Femoral Diaphysis and Femoral Neck

Group <sup>a</sup>	$F_u$	S	$\sigma_u$	E	U	$\epsilon_u$	Neck $F_u$
Baseline	177 <sup>o.lh</sup>	521 <sup>o.s.lh</sup>	318 <sup>o</sup>	9.00 <sup>o</sup>	5.60 <sup>o</sup>	0.038	1161 <sup>h</sup>
	5	11	9	0.23	0.28	0.001	3
Sham	175 <sup>o.lh</sup>	574 <sup>o.b.lh</sup>	309 <sup>o</sup>	9.62 <sup>o</sup>	5.09 <sup>o</sup>	0.036	1161 <sup>h</sup>
	5	13	9	0.22	0.28	0.001	3
OVX	142 <sup>s.b.lh</sup>	466 <sup>s.b.lh</sup>	249 <sup>s.h.lh</sup>	7.80 <sup>s.b.lh</sup>	3.98 <sup>s.b.lh</sup>	0.035	1151 <sup>h</sup>
	3	11	6	0.22	0.23	0.001	3
OVX8	213 <sup>o.s.bh</sup>	694 <sup>o.s.bh</sup>	309 <sup>o.b</sup>	9.43 <sup>o.b</sup>	5.19 <sup>o</sup>	0.036	1520 <sup>o.s.bh</sup>
	5	18	5	0.14	0.26	0.001	5
OVX40	247 <sup>o.s.bj</sup>	813 <sup>o.s.bj</sup>	332 <sup>o.s.bj</sup>	10.13 <sup>o.bj</sup>	5.27 <sup>o</sup>	0.034	1640 <sup>o.s.bj</sup>
	4	14	6	0.12	0.22	0.001	4
ANOVA	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.015	<0.0001

- <sup>a</sup> Femora diaphyses were examined by 3-point bending to measure the ultimate force ( $F_u$ ), stiffness (S), ultimate stress ( $\sigma_u$ ), Young's modulus (E), work to failure (U), and ultimate strain ( $\epsilon_u$ ). Femora necks were loaded to failure to measure the ultimate force ( $F_u$ ). Data are mean followed by SEM (n=23 to 33). Significant differences from baseline, Sham, OVX, OVX8, or OVX40 are depicted as "b", "s", "o", "l", or "h", respectively.

## Vertebrae

Vertebral strength, i.e., both structural and material biomechanical properties, were decreased by OVX and increased in a dose-dependent manner above sham and baseline levels by LY.

LY caused a small increase in body weight and uterine weight of OVX animals.

### Skeletal Effects of LY333334 in Ovariectomized Rats with Established Osteopenia (Nonclinical Pharmacology Report BN5-02)

#### METHODS

This study was carried out in a 'delayed-dosing intervention model', i.e., 6-month old SD rats that were ovariectomized and subsequently permitted to lose bone for 1 month. In most experiments treatment was carried out by daily s.c. administration of LY (rhPTH1-34) or synthetic PTH1-34, at doses between 0 and 80 ug/kg/day, for 3 months. Bone mass measurements were carried out by QCT (during in-life phase of study and at study termination), and histomorphometry and biomechanical analyses were carried out of bones removed at the end of the study.

#### RESULTS

##### Bone mass

QCT analysis of the proximal tibial metaphysis showed a decrease in BMC and BMD upon ovariectomy. Treatment with 0.1, 0.3, 1, 3, 10, 30 ug/kg/d of LY for 3 months restored BMD in a dose-dependent manner, to sham or above-sham levels at doses  $\geq 3$  ug/kg (Figure 2). The efficacy of LY continued to increase to the highest dose used of 80 ug/kg. X-area of the metaphysis was not affected by OVX or LY.

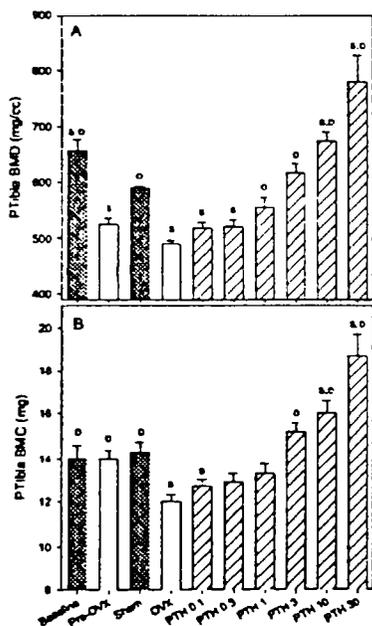


Figure 2

Dose-dependent effects of LY333334 in osteopenic ovariectomized rats. The proximal tibial metaphysis was analyzed by QCT, using voxel dimensions of 148 x 148 x 1200  $\mu\text{m}$ . Treatment was initiated after 1 month post-ovariectomy (7 months of age) and continued for the following 3 months. Groups included baseline, pretreatment OVX (Pre-OVX), Sham, OVX, and LY333334 at 0.1 to 30  $\mu\text{g}/\text{kg}/\text{day}$  subcutaneously, as indicated. Plotted data are mean  $\pm$  standard deviations with group sizes of n=7 to 8. Significant differences from Sham and OVX are depicted as "s" and "o", respectively ( $p < 0.05$ , Fisher's PLSD). LY333334 increased BMD and BMC dose dependently to significantly beyond OVX, Sham, and Baseline levels. (9721)

QCT of the femora showed qualitatively similar results, although the effects of OVX and LY, particularly in the midshaft area, were less pronounced.

#### Histomorphometry

Histomorphometry of the proximal tibial metaphysis showed some interesting results (Table 3).

**Table 3** Histomorphometry of LY333334 Effects on the Proximal Tibial Metaphysis

Group <sup>a</sup>	Bn.Pm	Ob.S/BS	Es/BS	Oc.S/BS	N.Oc/BS
Sham	36.6±1.9 <sup>o</sup>	0.73±0.18 <sup>o</sup>	4.30±0.23 <sup>o</sup>	1.03±0.11	0.235±0.024 <sup>o</sup>
OVX	11.1±1.5 <sup>s</sup>	8.68±2.14 <sup>s</sup>	7.63±1.21 <sup>s</sup>	2.61±0.57	0.618±0.147 <sup>s</sup>
PTH 0.03	6.8±0.9 <sup>s</sup>	7.89±1.68 <sup>s</sup>	9.30±1.50 <sup>s</sup>	3.73±0.83 <sup>s</sup>	0.835±0.173 <sup>s</sup>
PTH 0.3	9.5±1.4 <sup>s</sup>	7.93±2.22 <sup>s</sup>	9.30±1.65 <sup>s</sup>	3.57±0.90 <sup>s</sup>	0.758±0.181 <sup>s</sup>
PTH 3	25.9±3.3 <sup>so</sup>	7.33±2.41 <sup>s</sup>	8.10±0.51 <sup>s</sup>	2.28±0.34	0.487±0.071

- <sup>a</sup> The proximal tibial metaphysis was analyzed by histomorphometry following treatment. Groups included Sham, OVX, and LY333334 (PTH) at 0.03, 0.3, and 3 µg/kg/day subcutaneously. Parameters analyzed included cancellous bone perimeter (Bn.Pm), osteoblast surface (Ob.S/BS), eroded surface (Es/BS), osteoclast surface (Oc.S/BS), and osteoclast number (N.Oc/BS), as previously described (Parfitt et al. 1987). Data are mean ± standard errors with group sizes of n=7 to 9. Significant differences from Sham or OVX are depicted as "s", "so", respectively (p<0.05, Fisher's PLSD). (9607)

Bone perimeter was decreased by OVX, and further decreased by 0.03-0.3 µg/kg LY, but increased by 3 µg/kg LY. Bone turnover, as indicated by the one formation and the three resorption parameters in Table 3, was increased by OVX, as expected. LY at doses of 0.03-0.3 µg/kg appeared to stimulate resorption, while at 3 µg/kg it decreased resorption. Formation (Ob.S/BS) was not significantly affected but it should be noted that any effect on osteoblast surface per se will be masked by an effect on BS, particularly at the higher doses at which bone perimeter and BMD are increased.

#### Bone strength

Biomechanical analysis of the femur showed a slight decrease in femoral midshaft strength due to OVX, and a dose-dependent increase in femoral midshaft thickness and strength, and in femoral neck strength, after 3 months of treatment with LY at doses of 0.3-30 µg/kg (Table 4)

**Table 4** LY333334 Effects on the Biomechanical Properties of the Femora

Group <sup>a</sup>	t	F <sub>u</sub>	S	Neck F <sub>u</sub>
Sham	.664±0.010	163±5 <sup>o</sup>	457±11	108±8
OVX	.621±0.018	145±4 <sup>s</sup>	438±19	108±9
PTH 0.3	.643±0.011	160±6	464±37	113±5
PTH 3	.683±0.009 <sup>o</sup>	169±9 <sup>o</sup>	476±12	133±3 <sup>o</sup>
PTH 30	.906±0.027 <sup>so</sup>	196±10 <sup>so</sup>	601±21 <sup>so</sup>	149±9 <sup>so</sup>

- <sup>a</sup> LY333334 effects on the biomechanical properties of femora were evaluated by 3-point bending of the femoral midshaft and load to fracture of the femoral neck. Femora were isolated from ovariectomized rats that were dosed for 3 months, starting 1 month post-surgery. Groups included Sham, OVX, and human PTH (1-34) (LY333334) at 0.3, 3, and 30 µg/kg/day subcutaneously. Parameters analyzed for the femora diaphysis included cortical thickness (t), ultimate force (F<sub>u</sub>), and stiffness (S). In addition, femoral neck strength was analyzed in shear (F<sub>u</sub>). Data are mean followed by SEM (n=7 to 8). Significant differences from Sham or OVX are depicted as "s", "so", respectively (p<0.05, Fisher's PLSD). (9565/9568)

#### Skeletal

(Nonclinical Pharmacology Report BN5-09)

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The skeletal effects of human recombinant parathyroid hormone PTH (1-34) (LY333334) were evaluated in ovary-intact rats, as a follow-up to the 2-year oncogenicity study of LY333334 in male and female Fischer 344 rats.

**Study 9904 (F344 rats, age at treatment start 1.5 month, treatment duration 9 months, doses 0, 0.03, 0.3, 5, 30, 75 ukd):**

In 1.5 month old F344 rats, the BMD of the proximal tibial metaphysis increased 2-fold in the absence of drug treatment from 1.5 to 10.5 months of age (age effect). LY333334 (0.03-75 ug/kg/day) treatment for 9 months, from 1.5 months of age, induced a dose-dependent increase in proximal tibial BMD, from 3 months after treatment initiation, of up to ca. 40% at 75 ukd. This treatment also caused an increase in wet weight of the femora of up to ca. 20% at 75 ukd. QCT images of the femur showed that LY caused some thickening of the cortex associated with somewhat reduced marrow space. This is in contrast to the findings in the 2-year carcinogenicity study, where marrow space was completely lost and femoral bone geometry was altered after 2 years of 30 or 75 ukd of LY treatment. This suggests that these changes occurred after at least 9 month of treatment.

Over 9 months, femoral BMD was increased by ca. 35% in vehicle controls. LY (5, 30, 75 ukd) caused an additional femoral BMD increase of up to 10% in 75 ukd treated animals (Table 1).

At these doses LY caused increased femoral midshaft strength (Fu) and stiffness (S) (as compared to vehicle controls) but had no effect on ultimate displacement (i.e., brittleness). In the femoral neck Fu was increased by 30 and 75 ukd LY. Vertebral BMD and strength were also significantly increased by LY up to 30% at doses of 5, 30 and 75 ukd (Table 1). Some reduction of both femoral neck and vertebral BMD and or strength was seen at low doses of PTH (0.03 and 0.3 ukd), possible related to a decrease in ultimate strain. Mature, 10.5 month old F344 rats had substantially lower osteocalcin levels and PTH immunoreactivity compared to young animals. The former is consistent with an age-dependent reduction in osteoblastic activity. Dose-dependent elevation of osteocalcin levels by LY333334 of up to 120% at 75 ukd indicated stimulation of osteoblastic activity.

**BMD of femoral midshaft and lumbar vertebrae, after 9 months of treatment of 1.5 month old intact rats with LY333334**

Group/Dose	Femur midshaft				Lumbar vertebrae			
	BMD (mg/cc)	% change	BMC (mg)	% change	BMD (mg/cc)	% change	BMC (mg)	% change
Baseline	780	-	0.61	-	460	-	0.98	-
Vehicle	1080	-	1.15	-	557	-	1.77	-
0.03	1075	-1%	1.17	+1.7%	552	-1%	1.72	-3%
0.3	1075	-1%	1.16	+1%	556	+0%	1.77	0%
5	1105	+2%	1.17	+1.7%	615	+10%	1.94	+10%
30	1130	+5%	1.26	+10%	690	+24%	2.30	+30%
75	1180	+9%	1.35	+17%	714	+28%	2.51	+42%

**Study 9937 (SD rats, age at treatment start 1.5 months, treatment duration 3 months, doses 0, 0.3, 5, 30, 75 ukd):**

LY increased proximal tibial BMD and femoral midshaft BMD at 30 and 75 ug/kg/day. The effect was larger at the cancellous tibial site. Femoral diaphyseal and femoral neck strength were also increased by LY doses of 30 and 75 ug/kg/day. At 75 ukd, femoral midshaft ultimate displacement and strain were slightly decreased.

**Study 9907 (SD rats, age at treatment start 6 months, treatment duration 3 months, doses 0, 0.03, 0.1, 0.3, 1, 3, 30 ukd):**

LY increased proximal tibial BMD at 3 and 30 ukd, as in the younger animals (Study 9937). However, the mature animals appeared to be slightly more responsive (effect at lower dose, and larger effect) than the young animals. LY also increased femoral midshaft BMD at 3 and 30 ukd. The effect was larger at the cancellous tibial site. Femoral diaphyseal strength was not affected at doses up to 30 ukd, but femoral neck strength was increased by 30 ukd. At 3 and 30 ukd, femoral midshaft ultimate displacement and strain were slightly decreased. Vertebral BMD was increased at 30 ukd.

**Other results****Peak bone mass**

Longitudinal analyses suggested that intact SD and F344 female rats attain peak bone mass (BMD) in the proximal tibia at about 7 months of age, and in the femoral midshaft between 9 and 13 months of age.

**Intact vs. OVX rats**

With respect to the effect on proximal tibial BMD, mature intact SD rats (aged 6 months) were less responsive to LY than mature ovariectomized animals (aged 7 months). However, BMD in intact rats was larger than in OVX rats at every dose of LY tested.

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STUDIES ON EFFECT OF DOSING FREQUENCY**Pharmacokinetic Profile of Human Parathyroid Hormone (1-34) (LY333334) and Serum Chemistry in Rats After Anabolic or Catabolic Injection Protocols****Nonclinical Pharmacology Report R43-01****Summary**

Intermittent administration of human parathyroid hormone (PTH) (1-34) has been shown to increase bone mass in animals and humans. However, if given continuously, or if administered at high doses, a net catabolic response is observed. Different delivery protocols for PTH(1-34) were evaluated in this report in order to correlate serum biochemical and PTH(1-34) pharmacokinetic profiles with the anabolic or catabolic response in bone. Subcutaneous injection protocols for biosynthetic human PTH(1-34) (LY333334) which resulted in either an anabolic or catabolic response in bone, were administered to 100 g male rats, and serum was collected at different times after dosing. After a single, subcutaneous dose of LY333334 (80 µg/kg), which gave an anabolic bone response after 18 days of daily treatment, serum Ca rapidly decreased to a nadir (10 minutes), followed by return to baseline by 40 minutes. Serum phosphorous also decreased but the decline was slower (nadir at 60 to 120 minutes). Serum PTH(1-34) (determined using an immunoradiometric assay) rose rapidly to a peak (10 minutes) ( $18.6 \pm 4.2$  ng/mL) and then quickly declined to background levels (240 minutes). LY333334 (13.3 µg/kg/injection) given as 6 injections (10 minutes intervals), which produced an anabolic bone response after 18 days of daily treatment, gave a longer duration serum Ca and phosphorous depression with a lower nadir than the single dose regimen. Serum PTH(1-34) rose to  $2.5 \pm 0.6$  ng/mL 5 minutes after the first dose and gradually increased to  $5.6 \pm 0.7$  ng/mL 5 minutes after the last dose. Levels returned to baseline by 240 minutes after the first injection. When LY333334 (13.3 µg/kg/injection) was administered for 6 injections (1 hour intervals), a catabolic response was observed in bone after 18 days of treatment. Serum Ca and phosphorous decreased after each injection followed by an increase just prior to the next dose. Serum PTH(1-34) rose to a  $C_{max}$  5 minutes after each dose (2.2-3.8 ng/mL) and declined to 2-10% of the peak values prior to the next dose but remained above baseline until 420 minutes after the first dose. The response in bone to PTH(1-34) may be primarily determined by the length of time serum concentrations remain above baseline and only secondarily by the  $C_{max}$  obtained.

# The Frequency of Daily Injections Determines Induction of an Anabolic Response in Bone by Human Synthetic and Biosynthetic (LY333334) Parathyroid Hormone Fragment, hPTH 1-34, in Young Rats.

## Nonclinical Pharmacology Report CG3-02

### Summary

Parathyroid hormone (PTH), given once daily, stimulates bone formation to increase bone mass in a variety of rodent models and osteoporotic humans. When used to treat patients with osteoporosis, PTH must be given by once daily injection. In assessing the feasibility of alternate delivery systems, such as an electrophoretic patch, we found it was not technically possible to deliver an efficacious dose once daily. The purpose of this study was to assess the bone mass of young rats given either synthetic human parathyroid fragment, hPTH 1-34, or biosynthetic human parathyroid fragment, hPTH 1-34, LY333334, as multiple doses within either 1h or 6-8h periods, by daily subcutaneous (sc) injections for 12 or 18 days.

Results showed that when PTH, as a total daily dose of 80µg/kg/d, was given as 6 injections within 1h daily, for 12 or 18 days, PTH increased bone mass of proximal tibias equivalently to the significant increase measured after once daily injections in young rats. When PTH was given as 3 injections over an 8h day, at the same total daily dose as once daily injections (i.e. 80µg/kg/d), a complex response was observed. Bone mass, expressed as BMD, did not differ significantly from controls. However, the increase in bone mineral content and cross-sectional area resulted in an equivalent increase in resistance to fracture of the femur neck as the positive controls. When injections of PTH were increased to 6/day at the same daily dose of 80µg/kg/d over a 6-8h period for 18 days, PTH was catabolic and decreased bone mass. We concluded that, if an efficacious dose of PTH was to be given in multiple injections, these had to be delivered rapidly within a short time period, to induce an increase in bone mass. More prolonged exposure to the hormone, by multiple injections over a 6-8h period, resulted in significant loss of bone mass that more closely resembled the response observed when PTH is given by continuous sc infusion.

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### OTHER EFFICACY PHARMACOLOGY STUDIES

Several other studies with LY and combinations of LY with other products were carried out in rats, and some studies with LY were done in mice. Selected studies are briefly discussed in this review.

#### Rats

(Report CG3-8)

The anabolic effects of human PTH(1-34) at 8- ug/kg, given by s.c. injection for 12 days, were independent of the gender, breed and strain in normal and mutant disease-prone rats. The bones of all mutants had similar responses to hPTH1-34.

(Report BN5-03)

A raloxifene analogue had an additive effect on cancellous bone mass when given in combination with PTH(1-34). After discontinuation of PTH, the analogue prevented rapid bone loss that was observed in the controls.

(Report CG3-15)

After treatment with PTH(1-34) in combination with a raloxifene analogue (RA) of ovx rats, for 3 months, maintenance therapy with RA alone, reduced PTH, or the two together all resulted in reductions in the treatment-enhanced BMD. Thus, discontinuation of PTH treatment reverses the previously obtained increase in bone mass.

(Report CG1-16)

In ovx rats, discontinuation of PTH(1-34) therapy, rapidly results in a decrease in bone mass. Estrogen and raloxifene maintained bone mass after PTH withdrawal. These results apparently contradict the results from study CG3-15 (above).

#### Mice

(Report BN5-07)

Ovx mice from 9 different strains were treated with LY333334 (10 ug/kg) for 2 months. There were variable positive and negative effects of aging, ovariectomy, and LY333334 on total body or femoral BMC, and other parameters (body weight, lean fat mass), depending on mouse strain.

(Report W53-26)

In adult male mice PTH(1-34) two out of the five tested strains responded positively to PTH treatment with respect to femoral diaphyseal midshaft BMC and X-area. The study indicates mouse strain sensitivity of the bone formation effect by PTH.

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## SAFETY PHARMACOLOGY

Safety pharmacology studies with LY333334 were carried out in adult male rats (CV effects), female beagle dogs (CV effects), and adult male mice (CNS and behavioral effects). Summary reviews of these studies are included in this review.

Report	Title	In Vivo		In Vitro		Results
		Species, Strain, Gender, & Age	Dose Route	Assay Type	Tissue/Cell Line	
<b>Safety Pharmacology Study Reports</b>						
General Pharmacology Report 1	The Acute Cardiovascular Effects of LY333334 [PTH (1-34)] Administered Subcutaneously to Conscious Male Sprague Dawley Rats	Rats Sprague Dawley, male, adult	LY333334 3, 10, 30, 100, 300, & 1000 µg/kg single dose SC			The no-observed-effect level for cardiovascular changes (increased heart rate and decreased blood pressure) was 4.3 µg/kg of LY333334
General Pharmacology Report 2	An Acute Cardiovascular Toxicity Study With LY333334 Administered Subcutaneously in Conscious Instrumented Female Beagle Dogs	Dogs, Beagle, female, 12 to 21 months	LY333334 6 µg/kg single dose SC			The female beagle dog demonstrated a consistent and reproducible decrease in arterial pressure and increases in left ventricular inotropic state and heart rate after treatment with 6 µg/kg LY333334 that is consistent with compound-induced vasodilation and compensatory physiological homeostatic mechanisms
General Pharmacology Report 3	The Acute Behavioral Profile of LY333334 Following Subcutaneous Administration in Male CD-1 Mice	Mouse, CD-1, male, adult	LY333334 10, 30, & 100 µg/kg single dose SC			LY333334 at doses ≤100 µg/kg would not be expected to produce secondary pharmacology related to central nervous system (CNS) and behavioral functions such as changes in body temperature, ambulatory and nonambulatory activate levels, CNS depression, and convulsive thresholds

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**The Acute Cardiovascular Effects of LY333334 [PTH(1-34)] Administered Subcutaneously to Conscious Male Sprague Dawley Rats (Studies RV0595 and RV0795)**  
(General Pharmacology Report 1)

### METHODS

LY333334 was administered subcutaneously to conscious male Sprague Dawley rats (300-600g) at doses of 100, 300 or 1000 µg/kg (N=4/group) (Study RV0595), or nominal dose levels of 3, 10 and 30 µg/kg (actual levels 0.5, 4, 23 µg/kg) (Study RV0795). Cardiovascular parameters measured were heart rate, systolic, diastolic, and mean arterial pressure, and arterial pulse pressure. Data were acquired from 5h before to 4 h after dosing.

### RESULTS

Transient redness of extremities at all doses. Increase in heart rate and decrease in blood pressure (systolic, diastolic, and mean arterial pressure) at dose levels of 30 (real dose level 22.8), 100, 300, and 1000 µg/kg. Cardiovascular effects were observed during the first 2 hours after dosing. The no-observed-effect level was 4.3 µg/kg (nominal level 10 µg/kg). This dose is equivalent to approximately 1.5x the human exposure at a 20 µg daily dose.

### CONCLUSION

LY333334 has a vasorelaxing and hypotensive action and causes an increased heart rate in rats at doses ≥ 23 µg/kg.

**An Acute Cardiovascular Toxicity Study With LY333334 Administered Subcutaneously in Conscious Instrumented Female Beagle Dogs (Study DV0196)**  
(General Pharmacology Report 2)

**METHODS**

LY333334 (6 ug/kg) was administered subcutaneously to conscious female beagle dogs. Cardiovascular parameters measured were heart rate, left ventricular inotropic state, systolic, diastolic, and mean arterial pressure, and arterial pulse pressure. The peak value of the first derivative of left ventricular pressure (dP/dtmax) was used as an index of left ventricular inotropic state. Electrocardiograms were also recorded. Serum ionized calcium levels were determined approximately 6 hours after dosing

**RESULTS**

Decrease in arterial pressure (systolic, diastolic, mean arterial pressure, arterial pulse pressure), increase in left ventricular inotropic state, and increase in heart rate. All effects were maximal during the first 2 hours after dosing. Heart rate was statistically elevated during the first 6 hours after dosing. Serum ionized calcium levels were significantly elevated 6 hours after dosing. There were no significant treatment-related electrocardiographic effects. The increase in left ventricular inotropic state and heart rate could be due a reflex increase in sympathetic outflow in response to a decrease in total peripheral resistance due to vasodilation.

**CONCLUSION**

LY333334 causes vasodilation, hypotension and a compensatory increase in heart rate in female beagle dogs at 6 ug/kg.

**Acute cardiovascular effects of LY333334 in rats and dogs**

Species	Study type	Doses (ug/kg/day)	EFFECTS			
			Heart rate	Arterial blood pressure	Other findings	ECG findings
RAT (Sprague-Dawley), male	Single dose	0, 0.5, 4, 23, 100, 300, 1000	Increased at doses $\geq$ 23 ug/kg	Decreased at doses $\geq$ 23 ug/kg	Transient redness of extremities	-
DOG (Beagle), female	Single dose	0, 6	Increased	Decreased	Left ventricular inotropic state increased; Serum ionized Ca level elevated postdosing	No abnormalities

Thus, in rats and dogs, effects on heart rate and blood pressure were consistent with drug-induced vasodilation and a compensatory cardiac response.

**The Acute Behavioral Profile of LY333334 Following Subcutaneous Administration in Male CD-1 Mice (Studies PN9830 and PN9836)**  
(General Pharmacology Report 3)

**METHODS**

LY333334 was administered subcutaneously to CD-1 mice at doses of 0 (vehicle), 10, 30, or 100 ug/kg (10 males/treatment group).

**RESULTS**

No overt clinical signs or changes in body temperature at any dose examined. No changes in spontaneous ambulatory and nonambulatory activity levels. Hexobarbital-induced sleep times were not affected by administration of LY333334, indicating that LY333334 does not produce central nervous system (CNS) depression or inhibit hepatic enzymes involved in hexobarbital metabolism.

No changes in convulsive thresholds were observed as evaluated by the administration of electroshock or pentylenetetrazol.

**CONCLUSION**

LY333334 has no acute adverse effects on the central nervous system in the mouse at doses  $\leq 100$  ug/kg.

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## GENERAL TOXICOLOGY

### ACUTE TOXICITY STUDIES

#### Acute Toxicity Of LY333334 Administered Subcutaneously to F344 Rats (Study Nr. R07695)

(Toxicology Report 04)

(From IND Review September 25, 1995)

##### METHODS

Fischer 344 (F344/NHsd) rats (5/sex/dose group), age 9-10 weeks, weight 130-170 g, were fasted for 18 h, and then given a s.c. injection of 0, 100, 300, 1000 ug/kg, and observed for 14 days.

##### RESULTS

No deaths occurred. Redness of extremities was seen in all treated within 15 minutes after dosing, and was reversed within 2 h. Body weight gain was the same for controls and treated. No gross abnormalities were detected at necropsy. Median LD was >1000 ug/kg.

#### Acute Toxicity of LY333334 Administered Intravenously to F344 Rats (Study Nr. R07595)

(Toxicology Report 05)

(From IND Review September 25, 1995)

##### METHODS

Fischer 344 rats (5/sex/dose group), age 9-10 weeks, weight 130-170 g, were fasted for 17h, and then given an intravenous dose of 0, 300 ug/kg, and observed for 14 days.

##### RESULTS

No deaths occurred. Redness of extremities was seen in all treated immediately after dosing, and was reversed within 2 h. Body weight gain was the same for controls and treated. No gross abnormalities were detected at necropsy. Median LD was >300 ug/kg.

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## CHRONIC TOXICITY STUDIES

### A Chronic Toxicity and Blood Level Study in Rats given LY333334 by S.C. Injection for 6 Months (Study Nrs. R01196 and R04396)

(Toxicology Report 12).

(From IND Review February 7, 1997)

#### METHODS

Fischer 344 rats (n=15/sex/dose group), weight 200-450 gr, age 27-28 weeks, were administered, by subcutaneous injection, 0, 10, 30, 100 ug/kg/day (control, LD, MD, HD) LY333334. In the satellite toxicokinetic Study Nr. R04396, blood samples were taken on Day 0 from n= 3/sex (control), 6/sex (LD, MD), or 15/sex (HD), at 0h (pre-dose) in control group, at 0h and 0.25 h ( $T_{max}$ ) in LD and MD, and at 0-3h (10 samples) in HD. In the main toxicity Study Nr. R01196 samples were taken on Day 140 from n=15/sex (LD, MD, HD), at 0h and 0.25h in LD and MD, and at 0-3h (10 samples) in HD. Samples were assayed for immunoreactive PTH(1-34) by immunoradiometry. Testosterone and estradiol were also assayed by RIA.

#### RESULTS

##### **Clinical signs -**

In 2 MD f, and 1 HD f, blood in vaginal lavage sample during last 2 wks of treatment period. Vaginal bleeding in 1 HD f.

##### **Mortality -**

1 MD m killed on Day 170 due to hindleg weakness: upon necropsy, animal had spinal cord injury. 1 HD m died on Day 56 for unknown cause: animal had nasal/pulmonary hemorrhage. No drug-related mortality.

##### **Body Weight -**

###### *Males:*

After 6 months, significant decrease in body weight (BW) as compared to control in HD (0.96x control value); non-significant (n.s.) decrease in BW gain in HD (0.24x). After 3 months significant decrease in BW in HD (0.95x) and significant decrease in BW gain in HD (0.7x). In first 5 wks, slight decrease in BW in all groups including control.

###### *Females:*

After 6 months, treatment-related, significant increase in BW in LD, MD, HD (1.07x, 1.1x, 1.1x); increase in BW gain in LD (n.s.), MD, HD (1.4x, 1.7x, 1.8x). After 3 months significant increase in BW in MD, HD (1.05x, 1.05x) and increase in BW gain in MD (n.s.) and HD (1.5x, 1.65x). In first 2 wks, minimal decrease in BW in all groups including control.

##### **Food Consumption -**

###### *Males:*

Relative food consumption (RFC) (g/kg/day) significantly increased in HD in wks 12-26 (1.03-1.04x). EFU significantly decreased in HD in wks 5-26 (0.7x after 26 wks).

###### *Females:*

FC significantly increased in HD in wks 4-10 and wks 14-26 (1.06x after 26 wks). RFC significantly decreased in LD, MD, HD in wks 21-26 (0.96x, 0.95x, 0.97x after 26 wks). EFU significantly increased in LD, MD, HD in wks 22-26, 10-26, 3-26 respectively (1.4x, 1.6x, 1.7x after 26 wks)

##### **Vital Signs - No data.**

##### **Ophthalmoscopy - No compound-related effects.**

##### **Hematology - (significant changes) (samples taken at Month 1,3,6 from N= 10,10,15 or 14)**

###### *Males:*

Month 1: Minimal decreases in Hb conc, PCV, MCV and MCH in MD, HD. Slight decrease in