

### Figure Legends

**Fig. 1** Bone mineral density of the lumbar spine and tibia. The proximal metaphysis of the tibia (trabecular bone) is the upper 1/3 from the tibiofibular junction. The diaphysis of the tibia (cortical bone) is the middle 2/3 between the proximal epiphysis and the tibiofibular junction. Data show means  $\pm$  SE. Sham-operated Low calcium group (SL); Sham-operated Normal calcium group (SN); Ovariectomy Low calcium group (OL); Ovariectomy Normal calcium group (ON). \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs SN, <sup>#</sup>  $p < 0.05$ , <sup>###</sup>  $p < 0.001$  vs SL.

**Fig. 2** Calcium and deoxypyridinoline excretions in urine. Four balances were carried out to determine the 24 hr total calcium and deoxypyridinoline excretions in urine. Urine was collected at four phases. I : the 3rd and 4th days, II : the 31st and 32nd days, III: the 56th and 57th days, IV: the 80th and 81st days from the day of ovariectomy and the start of experimental diet (Low and Normal calcium diets). Data show means  $\pm$  SE. Sham-operated Low calcium group (SL); Sham-operated Normal calcium group (SN); Ovariectomy Low calcium group (OL); Ovariectomy Normal calcium group (ON). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs SN, <sup>#</sup>  $p < 0.05$ , <sup>##</sup>  $p < 0.01$  vs SL.

Fig 1.

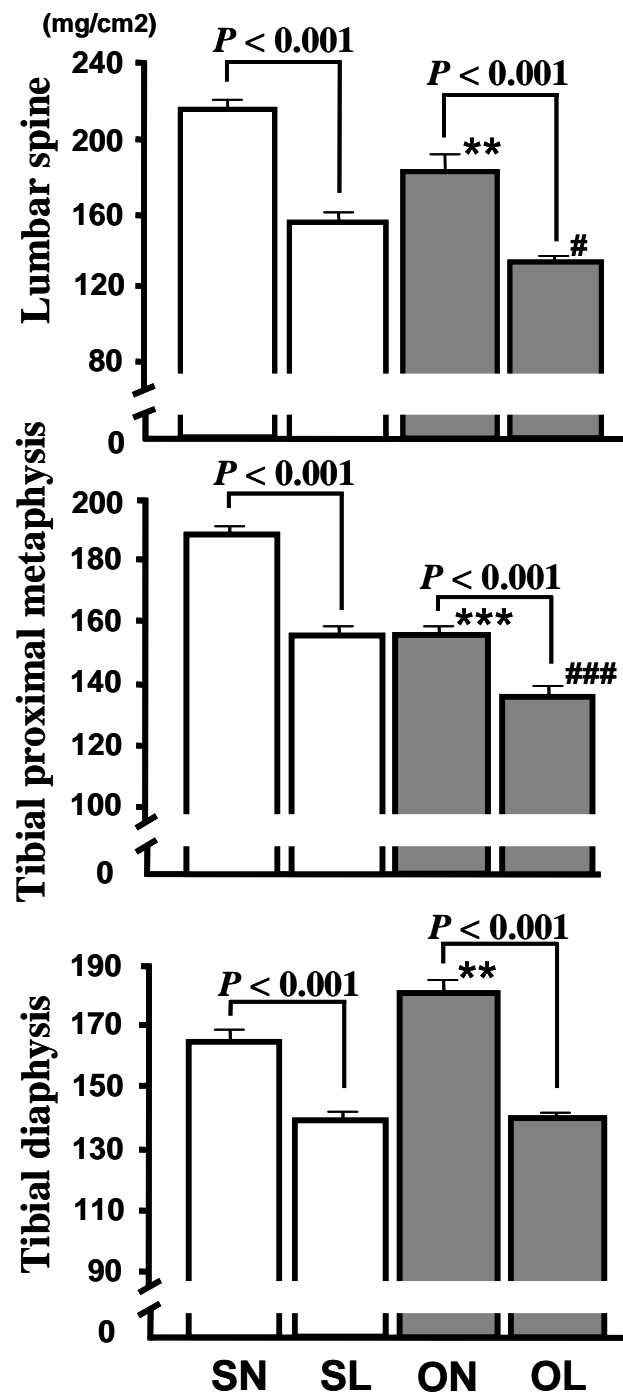
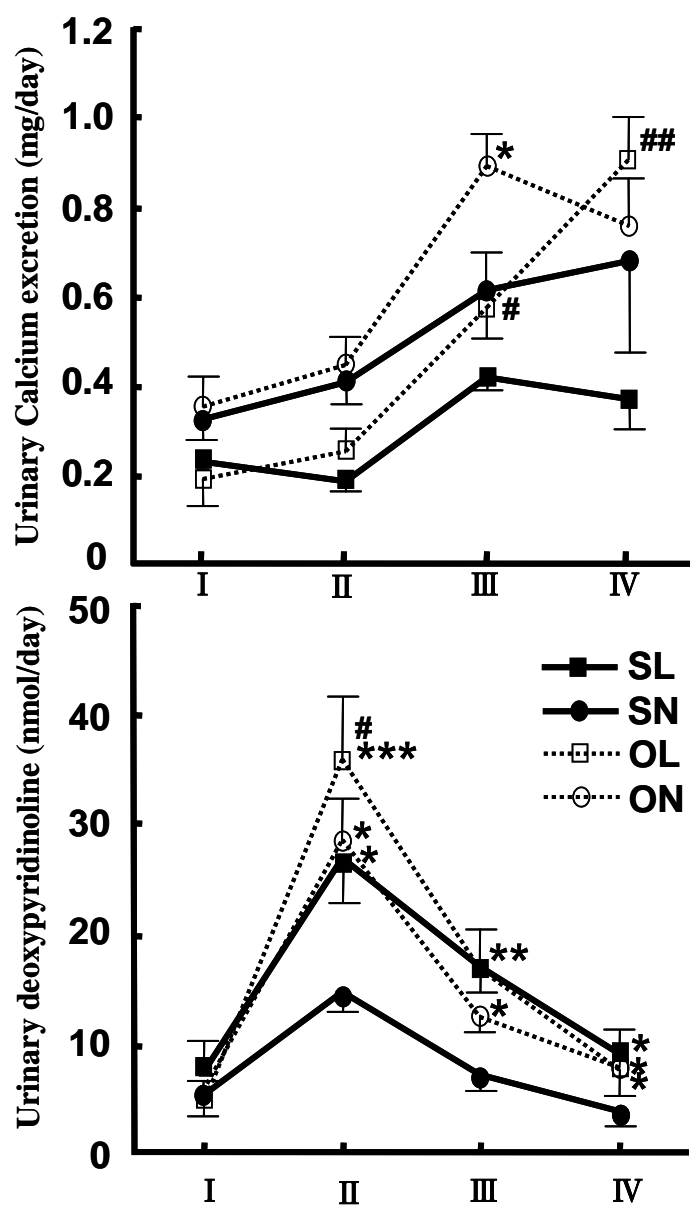


Fig 2.



**Table 1**

Body weight, food intake, and food efficiency

	Initial BW (g)	Final BW (g)	BW gain (g/day)	Food intake (g/day)	Food efficiency
<b>SN</b>	179.4 ± 1.6	321.8 ± 11.1	1.7 ± 0.1	16.2 ± 0.46	0.11 ± 0.01
<b>SL</b>	179.5 ± 2.0	329.3 ± 10.1	1.8 ± 0.1	16.4 ± 0.39	0.11 ± 0.01
<b>ON</b>	179.0 ± 2.8	427.8 ± 14.1 <sup>¶</sup>	3.0 ± 0.2 <sup>¶</sup>	19.4 ± 0.66 <sup>¶</sup>	0.16 ± 0.00 <sup>¶</sup>
<b>OL</b>	178.3 ± 2.7	432.8 ± 18.1 <sup>¶</sup>	3.1 ± 0.2 <sup>¶</sup>	19.3 ± 0.67 <sup>¶</sup>	0.16 ± 0.01 <sup>¶</sup>
Two- way ANOVA					
Ovariectomy		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Ca diet		0.9079	0.9089	0.6054	0.6531
Interaction		0.4381	0.4593	0.3941	0.4814

Values are means ± SE. Sham-operated Low calcium group (SL); Sham-operated Normal calcium group (SN); Ovariectomy Low calcium group (OL); Ovariectomy Normal calcium group (ON).

<sup>¶</sup>  $p < 0.001$  vs Sham-operated groups. BW, Body Weight

**Table 2**Serum calcium, phosphorus, bone turnover markers, and 1, 25-(OH)<sub>2</sub>D<sub>3</sub>

	Calcium (mg/dl)	Phosphorus (mg/dl)	BAP (U/l)	TRAP (U/l)	1,25-(OH) <sub>2</sub> D <sub>3</sub> (pg/ml)
<b>SN</b>	9.23 ± 0.11	5.47 ± 0.28	30.97 ± 1.09	30.69 ± 1.84	113.88 ± 11.25
<b>SL</b>	9.48 ± 0.09	5.70 ± 0.61	39.40 ± 3.43 *	25.39 ± 2.20	320.43 ± 34.28
<b>ON</b>	9.92 ± 0.16	6.22 ± 0.30	38.55 ± 3.62 *	31.14 ± 2.01	209.17 ± 24.64
<b>OL</b>	9.18 ± 0.23	5.95 ± 0.43	57.88 ± 2.92 <sup>†</sup>	37.96 ± 6.35 <sup>#</sup>	339.40 ± 47.24
Two- way ANOVA					
Ovariectomy	0.3467	0.2447	< 0.0001	0.2236	0.0643
Ca diet	0.3016	0.9718	< 0.0001	0.7471	< 0.0001
Interaction	0.0017	0.5361	0.0447	0.1314	0.2066

Values are means ± SE. BAP, Bone Alkaline Phosphatase activity; TRAP, Tartrate-resistant acid phosphatase activity. Sham-operated Low calcium group (SL); Sham-operated Normal calcium group (SN); Ovariectomy Low calcium group (OL); Ovariectomy Normal calcium group (ON). \*  $p < 0.05$  vs SN,

<sup>#</sup>  $p < 0.05$  vs SL, <sup>†</sup>  $p < 0.01$  vs the other groups

**Table 3**

Femoral characteristics and biomechanical testing

	Dry weight (g / 100g BW)	Ash weight (g / 100g BW)	Breaking force ( $\times 10^6$ dyn / 100g BW)	Breaking energy ( $\times 10^5$ erg / 100g BW)
<b>SN</b>	0.378 $\pm$ 0.013	0.239 $\pm$ 0.008	7.0 $\pm$ 0.3	3.3 $\pm$ 0.3
<b>SL</b>	0.330 $\pm$ 0.012**	0.196 $\pm$ 0.006***	5.4 $\pm$ 0.2***	2.5 $\pm$ 0.2*
<b>ON</b>	0.318 $\pm$ 0.008***	0.184 $\pm$ 0.004***	6.2 $\pm$ 0.2	3.0 $\pm$ 0.3
<b>OL</b>	0.284 $\pm$ 0.010 <sup>†</sup>	0.154 $\pm$ 0.008 <sup>†</sup>	4.1 $\pm$ 0.1 <sup>†</sup>	1.7 $\pm$ 0.2 <sup>†</sup>
Two- way ANOVA				
Ovariectomy	< 0.0001	< 0.0001	0.0015	0.1115
Ca diet	0.0006	< 0.0001	< 0.0001	0.0023
Interaction	0.4052	0.1245	0.4118	0.5675

Values are means  $\pm$  SE. Sham-operated Normal calcium group (SN); Sham-operated Low calcium group (SL); Ovariectomy Normal calcium group (ON); Ovariectomy Low calcium group (OL). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs SN, <sup>†</sup>  $p < 0.01$  vs the other groups. BW, Body Weight

**Table 4**

Thoracic and arch aortic wall composition

	Thoracic aorta			Arch aorta	
	Calcium	Elastin	Calcium	Elastin	Calcium
	(mg/g dry aorta)	(mg/g dry aorta)	(mg/g dry elastin)	(mg/g dry aorta)	(mg/g dry elastin)
<b>SN</b>	2.57 ± 0.37	336 ± 16	2.95 ± 0.45	338 ± 14	4.56 ± 0.87
<b>SL</b>	3.15 ± 0.72	346 ± 20	2.93 ± 0.55	331 ± 19	4.87 ± 0.65
<b>ON</b>	3.25 ± 0.84	350 ± 15	2.38 ± 0.25	323 ± 20	4.15 ± 0.97
<b>OL</b>	2.74 ± 0.61	339 ± 17	2.45 ± 0.21	322 ± 19	4.39 ± 1.53

Values are means ± SE. Sham-operated Low calcium group (SL); Sham-operated Normal calcium group (SN); Ovariectomy Low calcium group (OL); Ovariectomy Normal calcium group (ON).

**Table 5**

Tensile characteristics of thoracic aorta

	Incremental elastic modulus (g / mm <sup>2</sup> , at extension ratio 1.5 )	Ultimate tensile stress (g / mm <sup>2</sup> )	Ultimate tensile extension ratio
<b>SN</b>	23.38 ± 1.56	100.54 ± 3.77	2.92 ± 0.17
<b>SL</b>	24.48 ± 0.76	96.94 ± 1.87	2.80 ± 0.18
<b>ON</b>	24.56 ± 1.89	99.64 ± 4.16	3.28 ± 0.32
<b>OL</b>	24.12 ± 1.39	98.70 ± 5.79	3.13 ± 0.21

Values are means ± SE. Sham-operated Low calcium group (SL); Sham-operated Normal calcium group (SN); Ovariectomy Low calcium group (OL); Ovariectomy Normal calcium group (ON).