Estrogen deficiency and low-calcium diet increased bone loss and urinary calcium excretion but did not alter arterial stiffness in young female rats

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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 ± 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, $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ 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 ± 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, $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ vs SL.

Fig 1.

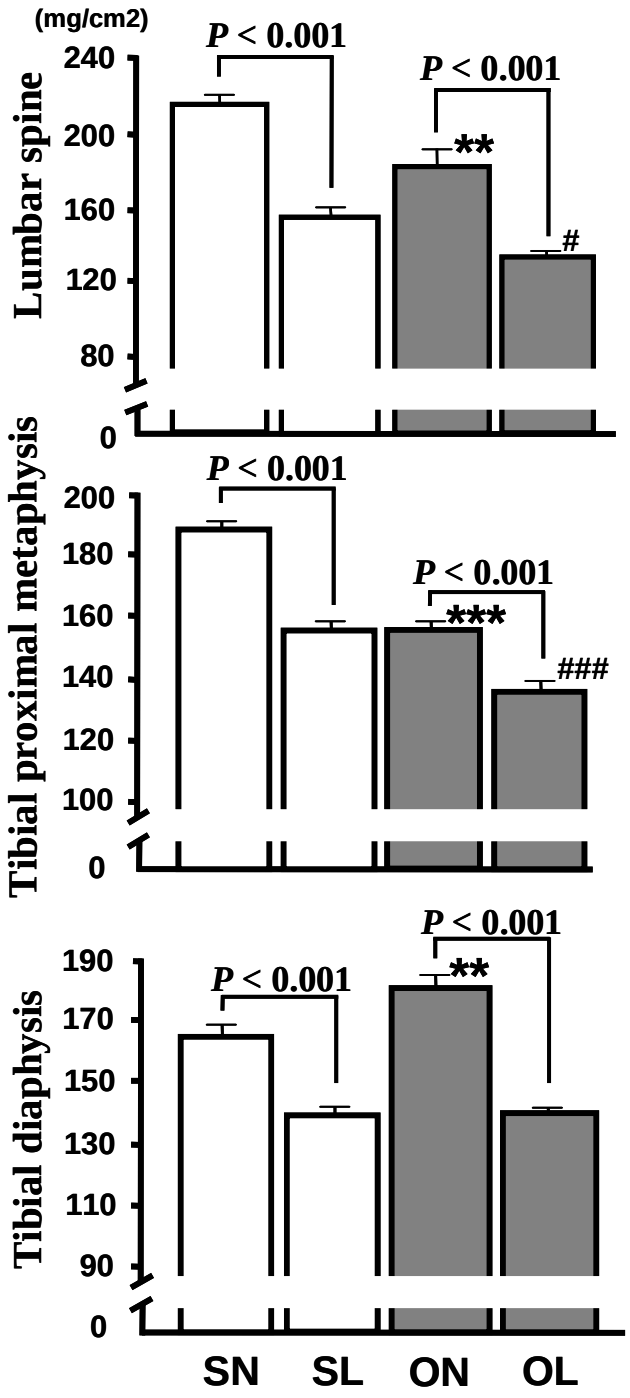
(mg/cm2)
Lumbar spine
P < 0.001
P < 0.001
**
#
240
200
160
120
80
0
Tibial proximal metaphysis
P < 0.001
P < 0.001
***
###
200
180
160
140
120
100
0
Tibial diaphysis
P < 0.001
P < 0.001
**
190
170
150
130
110
90
0
SN
SL
ON
OL

Fig 2.

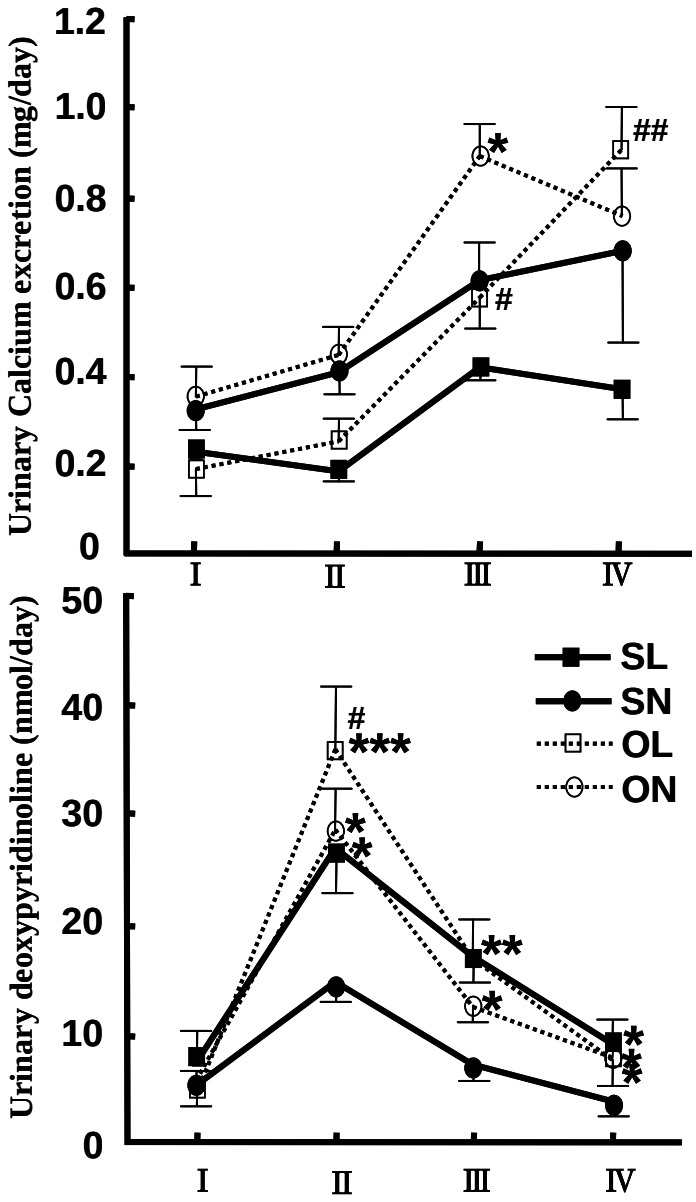
Urinary Calcium excretion (mg/day)
1.2
1.0
0.8
0.6
0.4
0.2
0
Ⅰ
Ⅱ
Ⅲ
Ⅳ
*
##
#
Urinary deoxypyridinoline (nmol/day)
50
40
30
20
10
0
SL
SN
OL
ON
#
***
**
*

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
SN	179.4 ± 1.6	321.8 ± 11.1	1.7 ± 0.1	16.2 ± 0.46	0.11 ± 0.01
SL	179.5 ± 2.0	329.3 ± 10.1	1.8 ± 0.1	16.4 ± 0.39	0.11 ± 0.01
ON	179.0 ± 2.8	427.8 ± 14.1 ¶	3.0 ± 0.2 ¶	19.4 ± 0.66 ¶	0.16 ± 0.00 ¶
OL	178.3 ± 2.7	432.8 ± 18.1 ¶	3.1 ± 0.2 ¶	19.3 ± 0.67 ¶	0.16 ± 0.01 ¶
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).
¶ $p < 0.001$ vs Sham-operated groups. BW, Body Weight

Table 2

Serum calcium, phosphorus, bone turnover markers, and 1, 25-$(OH)_2D_3$

	Calcium (mg/dl)	Phosphorus (mg/dl)	BAP (U/l)	TRAP (U/l)	1,25-$(OH)_2D_3$ (pg/ml)
SN	9.23 ± 0.11	5.47 ± 0.28	30.97 ± 1.09	30.69 ± 1.84	113.88 ± 11.25
SL	9.48 ± 0.09	5.70 ± 0.61	39.40 ± 3.43 *	25.39 ± 2.20	320.43 ± 34.28
ON	9.92 ± 0.16	6.22 ± 0.30	38.55 ± 3.62 *	31.14 ± 2.01	209.17 ± 24.64
OL	9.18 ± 0.23	5.95 ± 0.43	57.88 ± 2.92 †	37.96 ± 6.35 #	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, # $p < 0.05$ vs SL, † $p < 0.01$ 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)
SN	0.378 ± 0.013	0.239 ± 0.008	7.0 ± 0.3	3.3 ± 0.3
SL	0.330 ± 0.012**	0.196 ± 0.006***	5.4 ± 0.2***	2.5 ± 0.2*
ON	0.318 ± 0.008***	0.184 ± 0.004***	6.2 ± 0.2	3.0 ± 0.3
OL	0.284 ± 0.010†	0.154 ± 0.008†	4.1 ± 0.1†	1.7 ± 0.2†
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 ± 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, † $p < 0.01$ vs the other groups. BW, Body Weight

Table 4

Thoracic and arch aortic wall composition

	Thoracic aorta			Arch aorta	
	Calcium (mg/g dry aorta)	Elastin (mg/g dry aorta)	Calcium (mg/g dry elastin)	Elastin (mg/g dry aorta)	Calcium (mg/g dry elastin)
SN	2.57 ± 0.37	336 ± 16	2.95 ± 0.45	338 ± 14	4.56 ± 0.87
SL	3.15 ± 0.72	346 ± 20	2.93 ± 0.55	331 ± 19	4.87 ± 0.65
ON	3.25 ± 0.84	350 ± 15	2.38 ± 0.25	323 ± 20	4.15 ± 0.97
OL	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^2, at extension ratio 1.5)	Ultimate tensile stress (g / mm^2)	Ultimate tensile extension ratio
SN	23.38 ± 1.56	100.54 ± 3.77	2.92 ± 0.17
SL	24.48 ± 0.76	96.94 ± 1.87	2.80 ± 0.18
ON	24.56 ± 1.89	99.64 ± 4.16	3.28 ± 0.32
OL	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).