**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.
Fig 2.

- **Urinary deoxypyridinoline (nmol/day):**
  - Values range from 0 to 40.
  - Significant differences are indicated by asterisks: * for p < 0.05, ** for p < 0.01, *** for p < 0.001.
- **Urinary Calcium excretion (mg/day):**
  - Values range from 0 to 1.2.
  - Significant differences are indicated by asterisks: * for p < 0.05, ** for p < 0.01, *** for p < 0.001, ## for p < 0.001.

Legend:
- SL
- SN
- OL
- ON

Groups:
- I
- II
- III
- IV
### 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

| Source            | F-value | df | p-value  |
|-------------------|---------|----|----------|
| Ovariectomy       | < 0.0001| 1  | < 0.0001 |
| Ca diet           | 0.9079  | 1  | 0.6054   |
| Interaction       | 0.4381  | 1  | 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)₂D₃

|       | Calcium (mg/dl) | Phosphorus (mg/dl) | BAP (U/l) | TRAP (U/l) | 1,25-(OH)₂D₃ (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
- Ca diet: 0.3016
- Interaction: 0.0017

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 < 0.01 vs the other groups.
|        | Dry weight (g / 100g BW) | Ash weight (g / 100g BW) | Breaking force (× 10^6 dyn / 100g BW) | Breaking energy (× 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 | Ca diet | Interaction |
|----------------------|-------------|---------|-------------|
| Ovariectomy          | < 0.0001    | < 0.0001| 0.0015      |
| Ca diet              | 0.0006      | < 0.0001| < 0.0001    |

Interaction: 0.4052, Ca diet: 0.1245, Ovariectomy: 0.4118, Interaction: 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 / mm², at extension ratio 1.5) | Ultimate tensile stress (g / mm²) | 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).