

Figure legends

Figure 1. The calcium content, calcification, and elastic fiber network in the medial layer of aorta tissue. A: the arterial tissue calcium content in Sham-operated rats at 4 and 8 weeks after Control treatment (4SC and 8SC), Ovariectomized rats at 4 and 8 weeks after Control treatment (4OC and 8OC), Ovariectomized rats at 4 and 8 weeks after Vitamin D3 and Nicotine treatment (4OVN and 8OVN). B: von Kossa staining, showing linear and scattered aggregates of calcification (see arrows) among the elastic fibers of the aortas of 8SC, 8OC, and 8OVN. elastica van Gieson staining, exhibiting drastic disassociation and fragmentation of elastic fiber network (see triangles) in the medial layer of the aortas of 8SC, 8OC, and 8OVN (original magnification 40×). Values are expressed mean \pm SE.

Figure 2. The associations between arterial calcium content and indexes of bone loss, i.e., BMD of the tibial proximal metaphysis, femoral breaking force, and dry weight and ash weight of the femur, in sham-operated rats at 8 weeks after control treatment and ovariectomized rats at 8 weeks after Vitamin D3 and Nicotine treatment.

Figure 3. The endothelin-1 (ET-1) levels in the aortas of Sham-operated rats at 4 and 8 weeks after Control treatment (4SC and 8SC), Ovariectomized rats at 4 and 8 weeks after Control treatment (4OC and 8OC), Ovariectomized rats at 4 and 8 weeks after Vitamin D3 and Nicotine treatment (4OVN and 8OVN) (A). The relationship between arterial ET-1 level and calcium content in 8SC and 8OVN (B). Values are expressed mean \pm SE.

Figure 4. The endothelial nitric oxide synthase (eNOS) protein in aortas of Sham-operated rats at 4 and 8 weeks after Control treatment (4SC and 8SC), Ovariectomized rats at 4 and 8 weeks after Control treatment (4OC and 8OC), Ovariectomized rats at 4 and 8 weeks after Vitamin D3 and Nicotine treatment (4OVN and 8OVN). *Above*, typical examples of Western blotting analysis. The arrow indicates the Immunoblot band for eNOS protein (135kDa). Values are expressed mean \pm SE.

Figure 5. The typical examples of the expression of estrogen receptor α , β proteins in the aortas of Sham-operated rats at 4 and 8 weeks after Control treatment (4SC and 8SC), Ovariectomized rats at 4 and 8 weeks after Control treatment (4OC and 8OC), Ovariectomized rats at 4 and 8 weeks after Vitamin D3 and Nicotine treatment (4OVN and 8OVN) by Western blot. The arrow indicates the Immunoblot band for estrogen receptor α (66kDa) and β (54kDa).

Figure 1

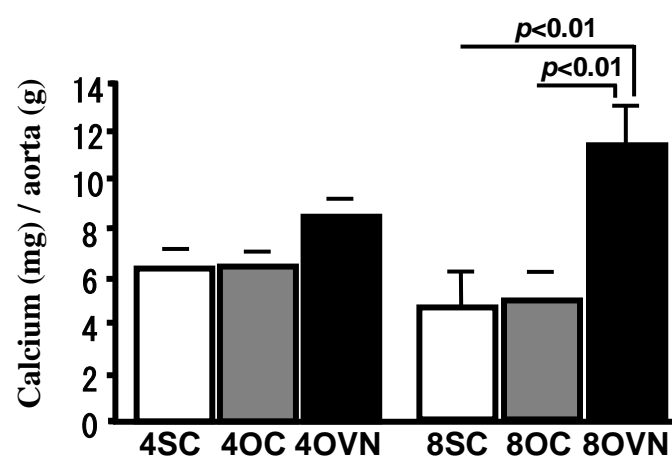


Figure 2

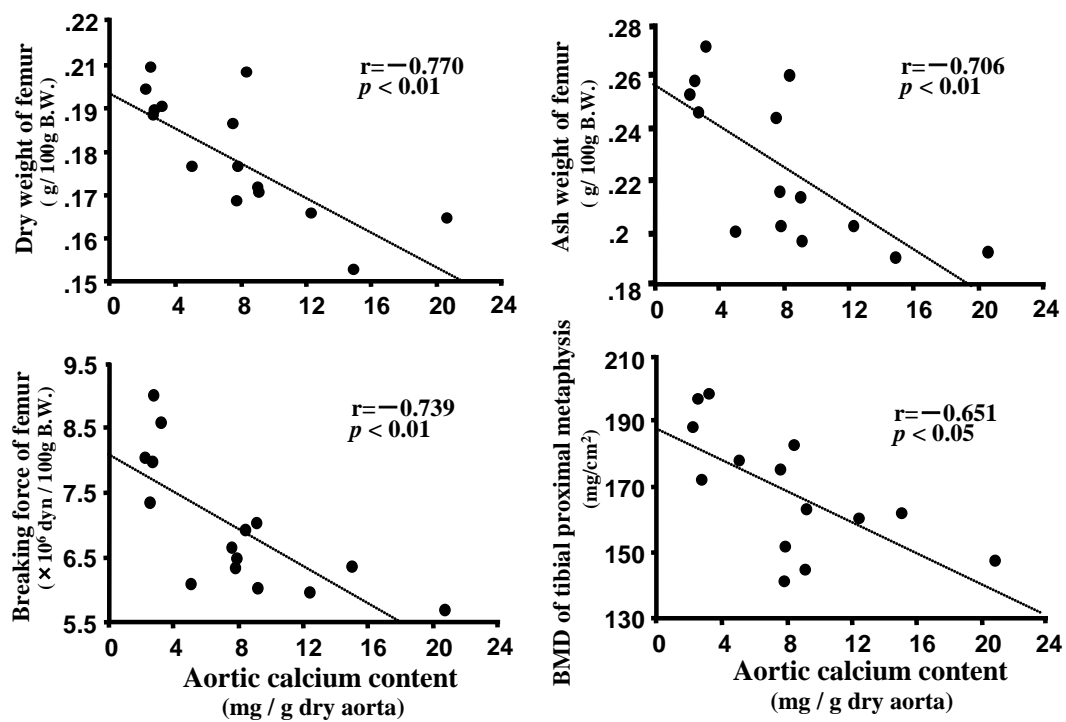


Figure 3

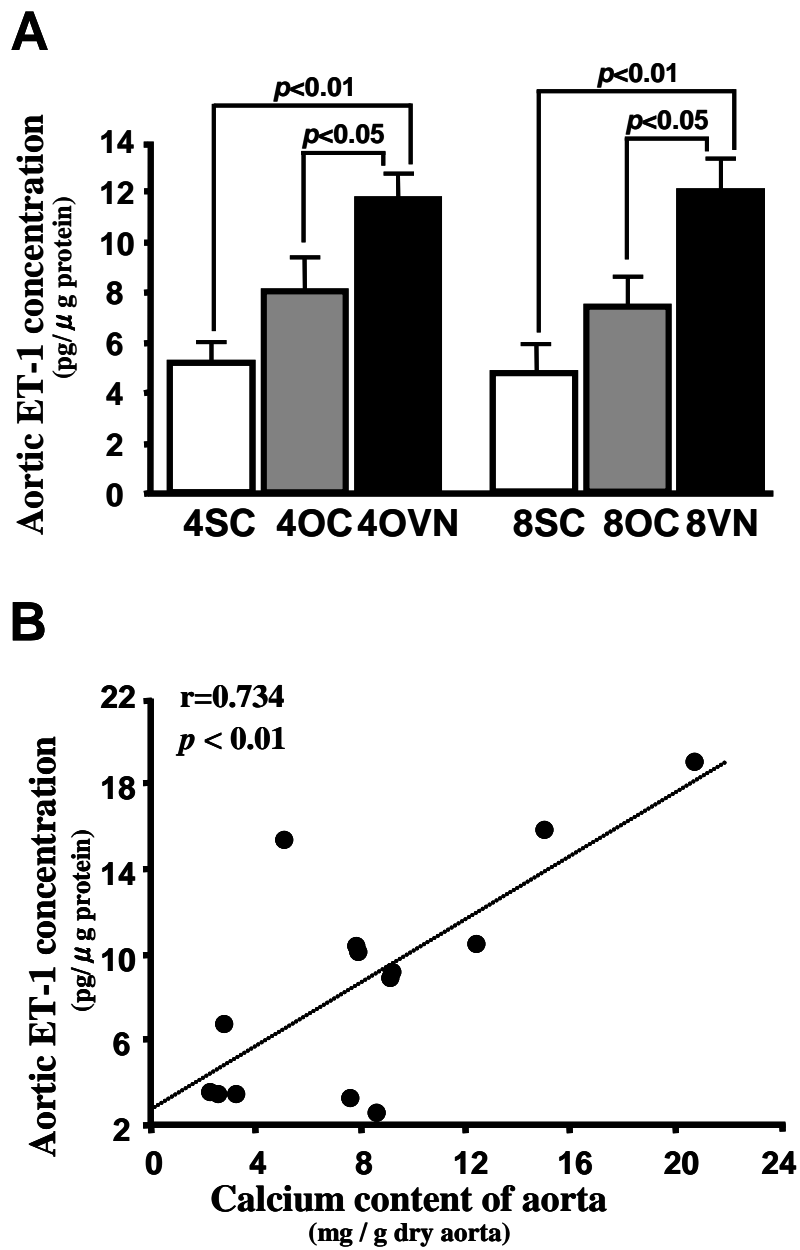


Figure 4

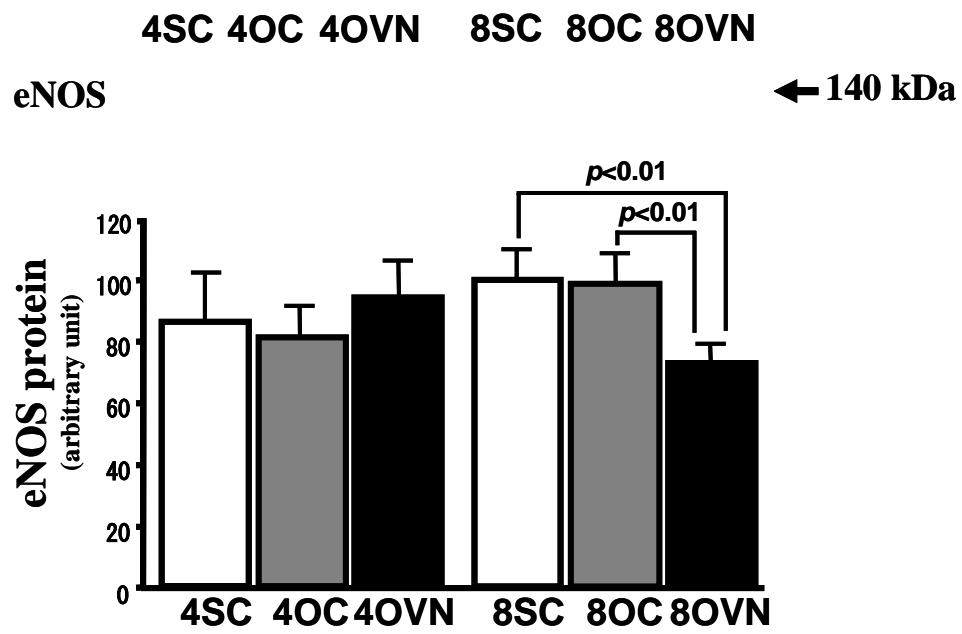


Figure 5

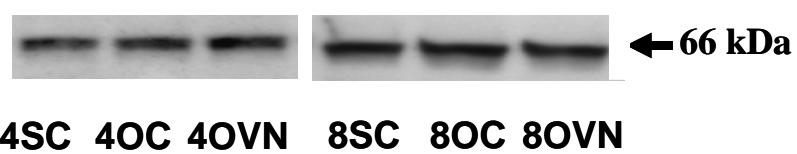
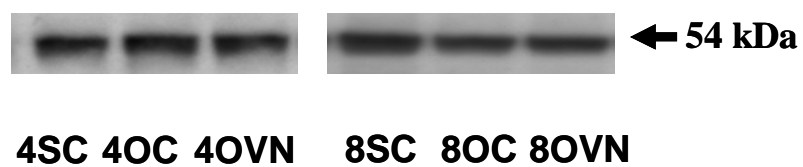
A**Estrogen α receptor protein****B****Estrogen β receptor protein**

Table 1. Plasma calcium and estradiol concentrations

	Calcium (mg/dl)	Estradiol (pg/ml)
4SC	9.58 ± 0.48	20.41 ± 0.88
4OC	9.79 ± 0.22	not detectable (below 7.8)
4OVN	9.61 ± 0.31	not detectable (below 7.8)
8SC	11.09 ± 0.43	18.08 ± 0.44
8OC	11.18 ± 0.27	not detectable (below 7.8)
8OVN	11.89 ± 0.35	not detectable (below 7.8)

Data show means ± SE. Sham-operated rats at 4 and 8 weeks after Control treatment (4SC and 8SC); Ovariectomized rats at 4 and 8 weeks after Control treatment (4OC and 8OC); Ovariectomized rats at 4 and 8 weeks after Vitamin D₃ and Nicotine treatment (4OVN and 8OVN). * $p < 0.001$, vs. the other groups.

Table 2. BMD of tibia and dry weight, ash weight, and mechanical test of femur

	Tibia	Femur		
	BMD of proximal metaphysis (mg/cm ²)	dry weight (g/100g B.W.)	ash weight (g/100g B.W.)	breaking force ($\times 10^6$ dyn / 100g B.W.)
4SC	158.90 \pm 7.82	0.185 \pm 0.003	0.121 \pm 0.004	7.360 \pm 0.43
4OC	150.84 \pm 2.80	0.154 \pm 0.004 ^{***}	0.095 \pm 0.002 ^{***}	6.806 \pm 0.27
4OVN	134.94 \pm 2.65 ^{†**}	0.148 \pm 0.003 ^{***}	0.092 \pm 0.002 ^{***}	5.970 \pm 0.20 ^{†***}
8SC	187.94 \pm 4.38	0.193 \pm 0.002	0.128 \pm 0.002	7.630 \pm 0.35
8OC	156.19 \pm 2.52 ^{***}	0.164 \pm 0.002 ^{***}	0.104 \pm 0.001 ^{***}	6.547 \pm 0.117 ^{**}
8OVN	154.85 \pm 4.63 ^{***}	0.169 \pm 0.002 ^{***}	0.101 \pm 0.002 ^{***}	6.262 \pm 0.143 ^{***}

Data show means \pm SE. Sham-operated rats at 4 and 8 weeks after Control treatment (4SC and 8SC); Ovariectomized rats at 4 and 8 weeks after Control treatment (4OC and 8OC); Ovariectomized rats at 4 and 8 weeks after Vitamin D₃ and Nicotine treatment (4OVN and 8OVN). ** $p < 0.01$, *** $p < 0.001$ vs SC, [†] $p < 0.05$ vs OC.