Supplementary materials

Table S1. Primer sequences for *Rattus norvegicus*.

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Table S2. Micro-computed tomography analysis of femoral bone in partially nephrectomized rats fed either a high or normal magnesium diet.

Table S1. Primer sequences for *Rattus norvegicus*.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	AGAAACCTGCCAAGTATGATGAC	TCATTGTCATACCAGGAAATGAG
RUNX2	GCCTTCAAGGTTGTAGCCCT	TGAACCTGGCCACTTGGTTT
ALPL	AGCTACACCACAACACGGG	CGTTGGTGTTGTACGTCTTGG
OCN	TCTGCTCACTCTGCTGGCC	TCAGAGTCGCTGGGCTTTG
ACTA2	ACCGGGAGAAAATGACCCAG	CCAGCACAATACCAGTTGTACG
SM22	CGATGGACACTACCGTGGAG	TTTGAAGGCCAATGACGTGC
KLF4	GACTAACCGTTGGCGAGAGG	GTAGGGCCGGGTTGTTACTG
Mycdn	CGATCAGTCTTACAGTTACGGC	CCTCGGGTCATGGAACTCAG
SRF	GATCCCTGTCTCTGCCGTTC	TTCACTCTTGGTGCTGTGGG

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; *RUNX2*, Runt-related transcription factor 2; *ALPL*, Alkaline phosphatase; *OCN*, Osteocalcin; *ACTA2*, Actin alpha 2, smooth muscle; *SM22*, Transgelin (smooth muscle 22 protein); *KLF4*, Kruppel-like factor 4; *Mycdn*, Myocardin; *SRF*, Serum response factor.

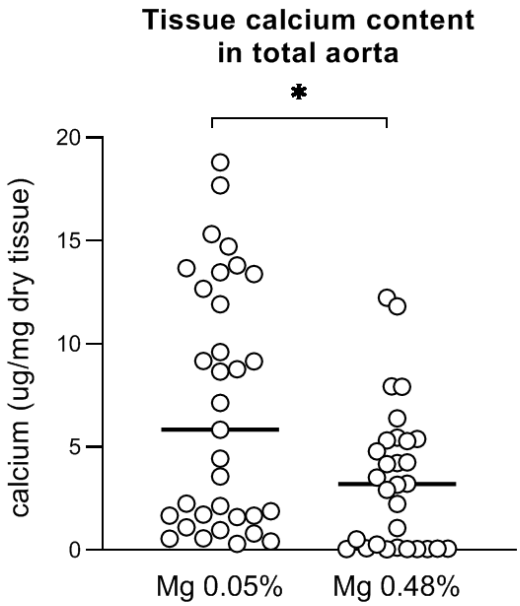

Figure S1. Calcium content in all aorta segments of partially nephrectomized rats fed either a high or normal magnesium diet. Dots represent the aortic segments of the animals. Tissue calcium content is expressed in μg per mg dry tissue. Horizontal lines represent the medians. Mg 0.05%, magnesium 0.05% (w/w) diet; Mg 0.48%, magnesium 0.48% (w/w) diet. *, $P<0.05$ for high vs. normal dietary magnesium.

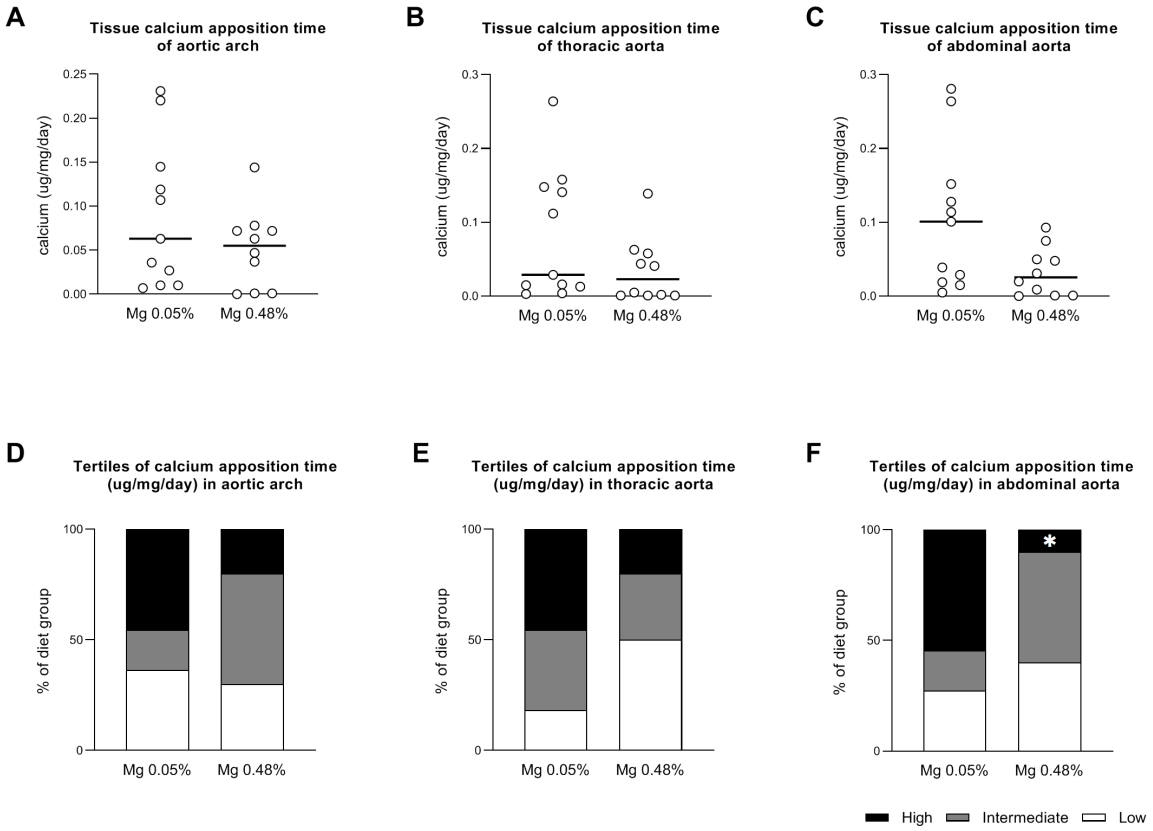

Figure S2. Calcium apposition time in aortic segments of partially nephrectomized rats fed either a high or normal magnesium diet. A-C. Calcium apposition time in μg per mg dry tissue per day in the aortic arch (A), thoracic (B) and abdominal aorta (C). Dots represent individual animals. Horizontal lines represent medians. **D-F.** The percentage of animals in each diet group, with a calcium apposition time within respectively the highest (black bars), intermediate (grey bars) and lowest tertile (white bars). Mg 0.05%, magnesium 0.05% (w/w) diet; Mg 0.48%, magnesium 0.48% (w/w) diet. *, $P<0.05$ for high vs. normal dietary magnesium.

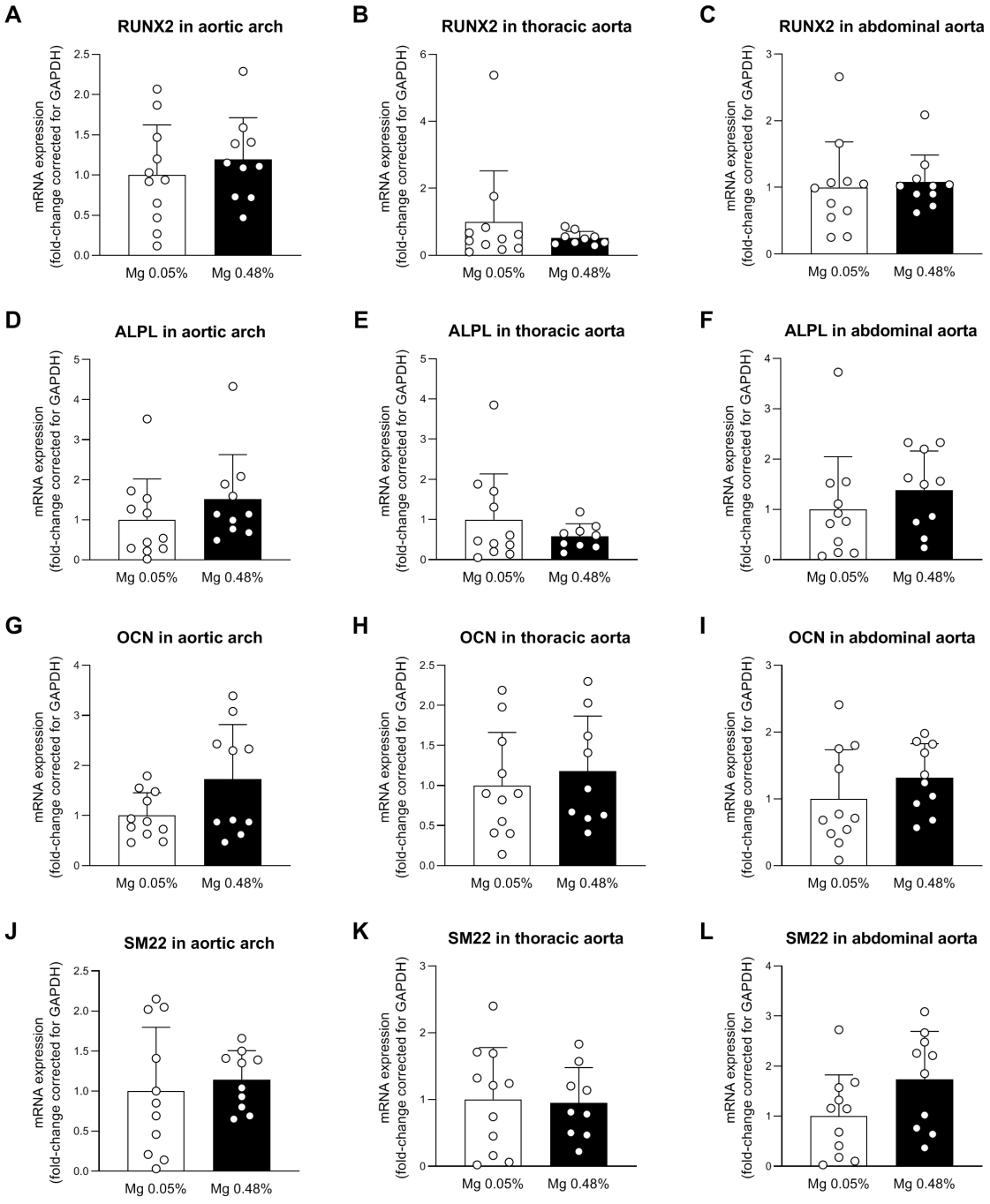

Figure S3. (Continued on next page)

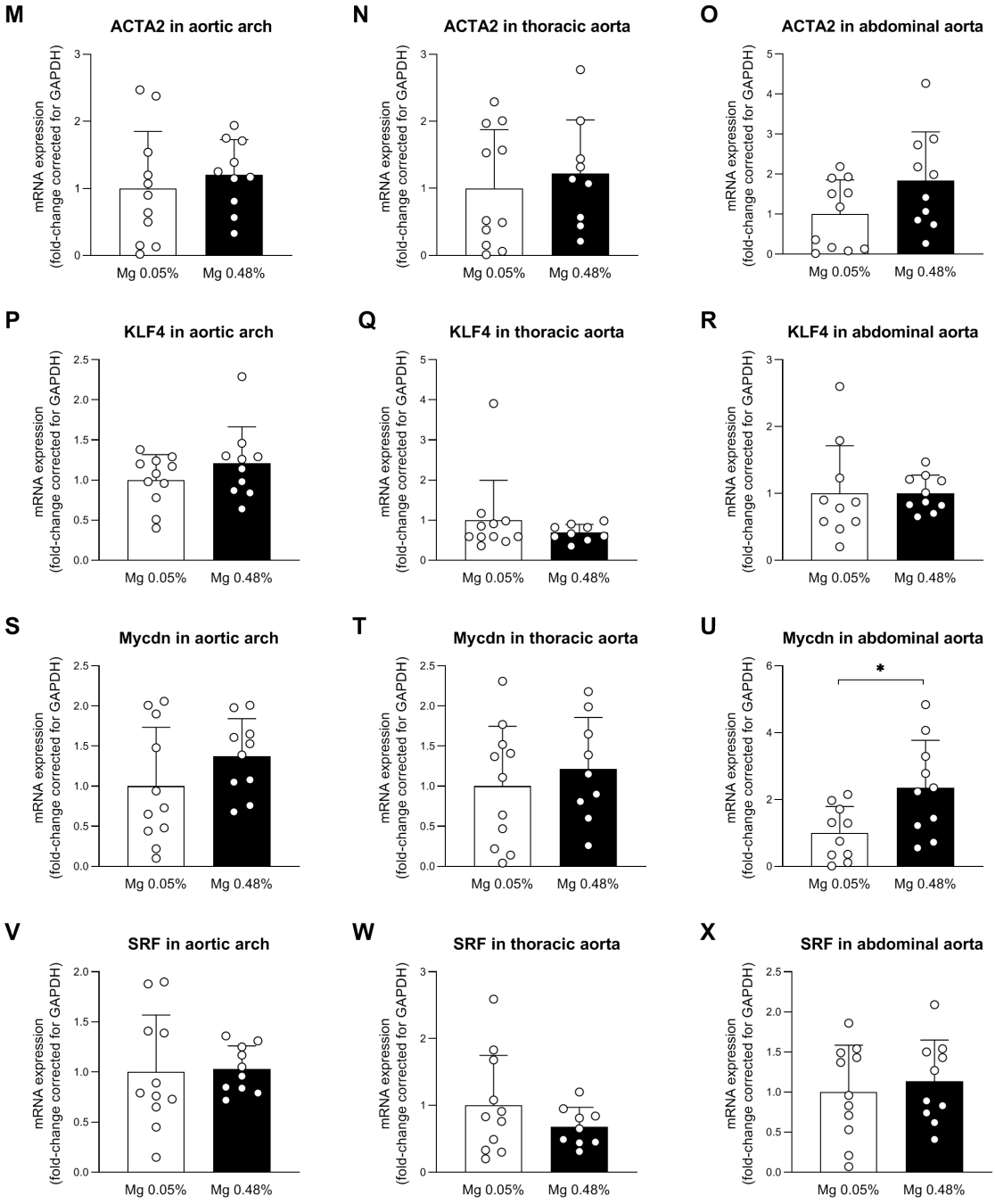

Figure S3. Messenger RNA expression of osteochondral and smooth muscle differentiation related genes in aortic segments of partially nephrectomized rats fed either a high or normal magnesium diet. Expression is shown as fold-change normalized to *GAPDH* for the high versus low dietary magnesium group. Expression of osteochondrogenic markers *RUNX2* (A,B,C), *ALPL* (D, E, F) and *OCN* (G, H, I), smooth muscle markers *SM22* (J, K, L) and *ACTA2* (M, N, O), and contractile phenotype markers *KLF4* (P, Q, R), *Mycdn* (S, T, U) and *SRF* (V, W, X) are shown in respectively the aortic arch, thoracic aorta and abdominal aorta. Dots represent individual animals. Bars represent means, whiskers represent standard deviations. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase; *RUNX2*, Runt-related transcription factor 2; *ALPL*, Alkaline phosphatase; *OCN*, Osteocalcin; *ACTA2*, Actin alpha 2, smooth muscle; *SM22*, Transgelin (smooth muscle 22 protein); *KLF4*, Kruppel-like factor 4; *Mycdn*, Myocardin; *SRF*, Serum response factor. Mg 0.05%, magnesium 0.05% (w/w) diet; Mg 0.48%, magnesium 0.48% (w/w) diet. *, P<0.05 for high vs. normal dietary magnesium.

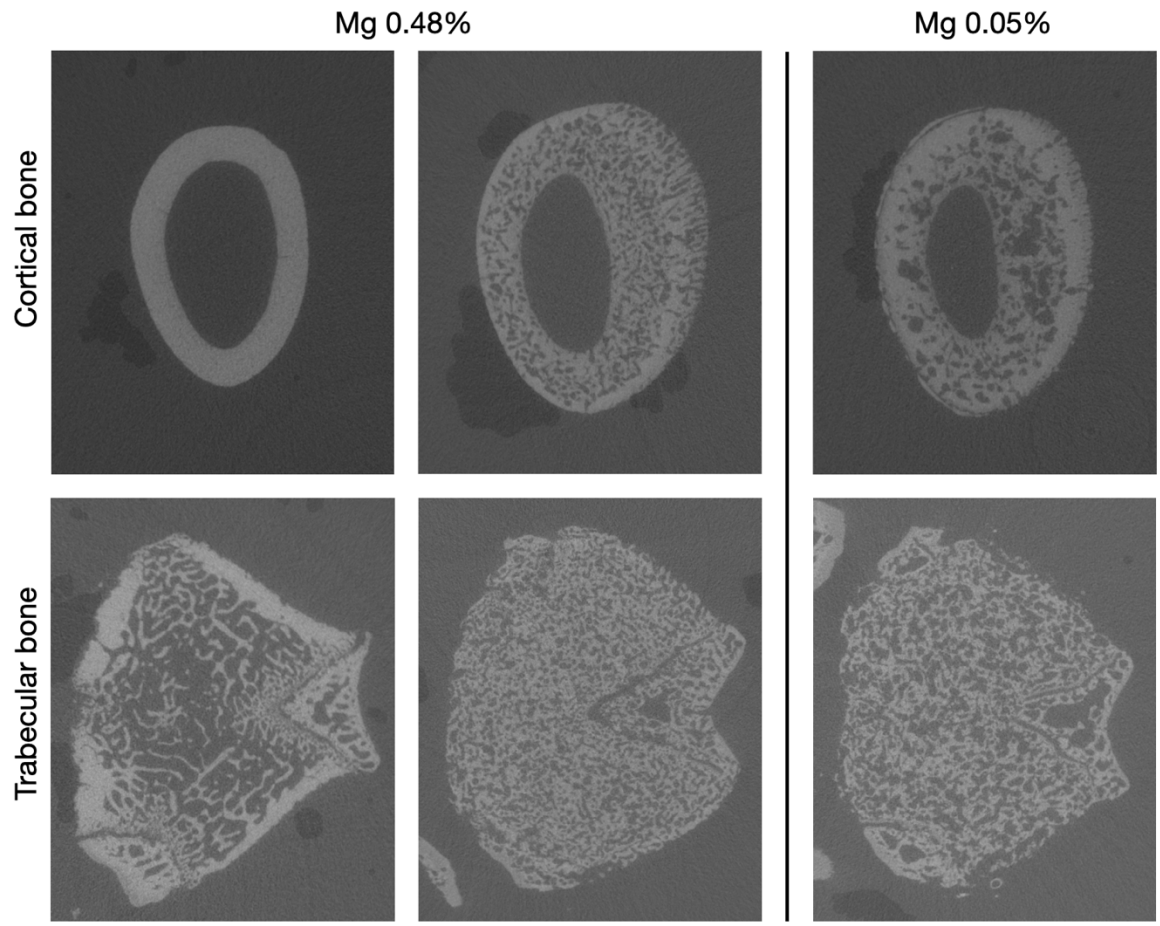

Figure S4. Micro-computed tomography (micro-CT) images of right femora of partially nephrectomized rats fed either a high (0.48%) or normal (0.05%) magnesium diet. Femur analysis was performed in all animals that completed a study period of at least 12 weeks. Right femurs were dissected, fixed in formalin 10% (v/v) for 24 hours, then stored in ethanol 70% (v/v) at 4°C, and transferred to phosphate buffered saline prior to analysis. *Ex vivo* micro-CT images were acquired at 70 kV, 114 mA and 9 um voxelsize with Scanco μCT 40 (Scanco Medical AG, Switzerland). Representative images of transverse cross-sections are shown from the mid-shaft for cortical bone, and from the metaphysis for trabecular bone. Images show cortical and trabecular bone with normal appearance in rats fed the Mg 0.48% diet, as was identified in 43% of animals in this group (left), and high cortical porosity or cortical trabecularization and dense trabecular bone with abnormal appearance, as was identified in the other animals of the Mg 0.48% group (middle) and all animals in the Mg 0.05% group (right).

Table S2. Micro-computed tomography analysis of femoral bone in partially nephrectomized rats fed either a high or normal magnesium diet.

	0.05% Mg diet *n=6*	0.48% Mg diet *n=7*	p-value
Femur length (mm)	38 ± 5	42 ± 4	0.08
Trabecular bone			
Bone mineral density, BMD (g/cm^2)	0.58 ± 0.06	0.38 ± 0.14	0.01
Bone volume fraction, BV/TV (%)	57 ± 6	35 ± 17	0.02
Trabecular separation, Tb.Sp (mm)	0.11 ± 0.01	0.21 ± 0.18	0.22
Trabecular thickness, Tb.Th (mm)	0.10 ± 0.01	0.07 ± 0.01	<0.01
Trabecular number, Tb.N (1/mm)	8.1 ± 0.7	6.8 ± 3.7	0.47
Cortical bone			
Tissue mineral density, TMD (g/cm^2)	1.09 ± 0.04	1.07 ± 0.10	0.74
Total area inside periostial envelope, Tt.Ar (mm^2)	16 ± 3	20 ± 9	0.34
Cortical bone area, Ct.Ar (mm^2)	12 ± 2	16 ± 11	0.43
Cortical area fraction, Ct.Ar/Tt.Ar (%)	76 ± 6	72 ± 20	0.66
Cortical thickness including pores, Ct.Th.iPo (mm)	1.05 ± 0.15	1.11 ± 0.51	0.80
Cortical thickness bone only, Ct.Th.ePo (mm)	0.15 ± 0.03	0.28 ± 0.21	0.19
Porosity fraction, Po.V/Ct.V (%)	26 ± 7	22 ± 22	0.72

Micro-computed tomography images were analyzed with Scanco's software using a gaussian filter (sigma 0.8, support 1) and segmentation with global thresholding (220-1000 for trabecular, 320-1000 for cortical) for quantification of mineral density and trabecular morphological parameters. Cortical morphological parameters were quantified in 3D analysis with CTAn software (version 1.20.8.0, Bruker Corporation, Billerica, MA, USA) with segmentation using visual global thresholding. For trabecular analyses, 200 slices of metaphyseal bone were used starting at an offset of 100 slices from the growth plate, and trabecular bone was delineated from cortical bone by manual contouring. For cortical bone, 200 slices of midshaft diaphyseal bone were analyzed. Variables are expressed as mean ± standard deviation. Mg, magnesium; BV, bone volume; TV, total volume; Ct.Th.iPo, cortical thickness defined as mean separation of periosteum and endostium; Ct.Th.ePo, cortical thickness defined as average local thickness, taking account of pores; Po.V, pore volume; Ct.V, cortical volume.