

Supplements

Supplemental Tables

Table S1. Serum Ca, phosphate, creatinine and alkaline phosphatase were measured weekly in group 1 (n=2-5), group 2 (n=1-5) and group 3 (n=2-4 animals per measurement). Serum Ca, phosphate creatinine, and alkaline phosphatase were in the normal range for rats throughout [49].

	Baseline (Group 1)	Standard-Ca-diet (Group 2)	Low-Ca-diet (Group 3)
Calcium (mmol/l)	2.84 ± 0.03	2.78 ± 0.12	2.70 ± 0.10
Phosphate (mmol/l)	3.9 ± 0.4	2.69 ± 0.6	2.9 ± 0.3
Creatinine (mg/dl)	0.42 ± 0.10	0.27 ± 0.10	0.24 ± 0.06
Alkaline phosphatase (U/l)	472 ± 57	397 ± 150	400 ± 102

Table S2. Tibia μ CT studies were performed after 2 weeks of standard-Ca-diet (0.5% Ca; baseline group 1), after a total of six weeks of standard-Ca-diet (group 2) and in rats after two weeks of standard-Ca-diet followed by 4 weeks of a low-Ca-diet (0.25%; group 3).

Right tibia		Baseline Week 2 (Group 1; n=10)	Standard-Ca-diet Week 6 (Group 2; n=19)	Low-Ca-diet Week 6 (Group 3; n=20)	p-value ^a
Volume (mm ³)	Total bone	301 \pm 20	406 \pm 22	373 \pm 24	.0002
	Δ tibia volume ^b		105 \pm 22	72.0 \pm 24	.0002
	Cortical bone	201 \pm 12	305 \pm 14	275 \pm 15	<.0001
	Cancellous bone	100 \pm 15	101 \pm 9.6	98 \pm 11	.41
	Diaphysis ^c	12 \pm 0.4	17.9 \pm 1.3	16.9 \pm 0.7	.004
	Metaphysis ^c	44 \pm 4.2	30.4 \pm 2.4	25.5 \pm 3.4	<.0001
	Epiphysis ^c	7.6 \pm 1.6	14.1 \pm 2.8	13.1 \pm 1.8	.18
Bone mineral density (HU)	Total bone HU	3791 \pm 108	4043 \pm 64	3992 \pm 73	.03
	Δ HU ^b		252 \pm 64	201 \pm 73	.03
	Cortical bone HU	2819 \pm 108	3086 \pm 64	3041 \pm 71	.05
	Cancellous bone HU	972 \pm 7.0	957 \pm 5.5	951 \pm 5.1	.002
	Diaphysis HU ^c	3655 \pm 162	3667 \pm 122	3581 \pm 101	.02
	Metaphysis HU	2586 \pm 53	2945 \pm 52	2875 \pm 67	.001
	Epiphysis HU ^c	2714 \pm 59	2885 \pm 64	2793 \pm 50	<.0001
Δ Hounsfield Units \times tibia volume (HU \times mm ³)			101341 \pm 21862	74059 \pm 24955	.001

a = G2 vs. G3, t-test; b = in the period of week 3-6; c = cortical + cancellous bone

Table S3. In group 1, six additional female rats were compared to the 12 male animals. All animals received 0.5% Ca diet for 2 weeks. Dietary food and thus Ca intake were reduced in female rats entailing an also reduced net body Ca uptake. None of the Ca isotope ratios in the female rats differed significantly from the male animals of group 1.

		Week 1/2		p-value ^a			
		female	male				
Ca balance	Food intake (g/day)	15.4 ± 0.4	22.1 ± 1.0	.001			
	Dietary Ca intake (mg/day)	76.8 ± 1.8	111 ± 5.0	.001			
	Fluid intake (ml/day)	22.9 ± 0.8	32.2 ± 1.2	<.0001			
	Fluid Ca intake (mg/day)	2.5 ± 0.1	3.5 ± 0.1	<.0001			
	Feces (g/day)	1.1 ± 1.1	2.8 ± 0.7	.001			
	Fecal Ca loss (mg/day)	12.4 ± 6.7	12.9 ± 6.4	.25			
	Intestinal Ca absorption (%)	90.5 ± 3.9	88.6 ± 5.6	.65			
	Intestinal Ca absorption (mg/day)	71.5 ± 3.1	100.9 ± 6.4	.002			
	Urine excretion (ml/day)	8.1 ± 4.0	8.5 ± 3.2	.83			
	Urine Ca excretion (mg/day)	1.0 ± 0.7	0.7 ± 0.4	.74			
Net body Ca uptake (mg / day)		70.6 ± 2.7	100.4 ± 6.6	.002			
μCT	Tibia	249 ± 13	301 ± 20	<.0001			
	Volume (mm ³)	Cortical bone	177 ± 8	201 ± 12	<.0001		
		Cancellous bone	71 ± 6	100 ± 15	<.0001		
	Tibia	3977 ± 21	3791 ± 108	.001			
	Bone density (HU)	Cortical bone	3004 ± 21	2819 ± 108	.001		
		Cancellous bone	973 ± 2	972 ± 7	.68		
δ ^{44/42} Ca (‰)	Femur	0.11 ± 0.04	0.15 ± 0.05	.34			
	Lower ribs	0.14 ± 0.10	0.13 ± 0.05	.86			
	Tibia: diaphysis	0.14 ± 0.05	0.18 ± 0.06	.14			
	Tibia: metaphysis	0.28 ± 0.05	0.27 ± 0.07	.94			
	Tibia: epiphysis	0.20 ± 0.06	0.26 ± 0.04	.11			
		Week 1		p-value ^a	Week 2		p-value ^a
		female	male		female	male	
Serum Ca (mmol/l)	Serum	0.47 ± 0.07	0.52 ± 0.03	.06	0.56 ± 0.03	0.58 ± 0.05	.54
	Feces	0.78 ± 0.06	0.88 ± 0.17	.39	0.95 ± 0.11	1.04 ± 0.12	.38
	Urine	1.19 ± 0.51	1.16 ± 0.54	.92	1.58 ± 0.21	1.54 ± 0.28	.84
Serum Ca (mmol/l)		3.06 ± 2.36	2.86 ± 0.73	.83	2.67 ± 0.27	2.82 ± 0.36	.87

a = t-test

Supplemental Figures

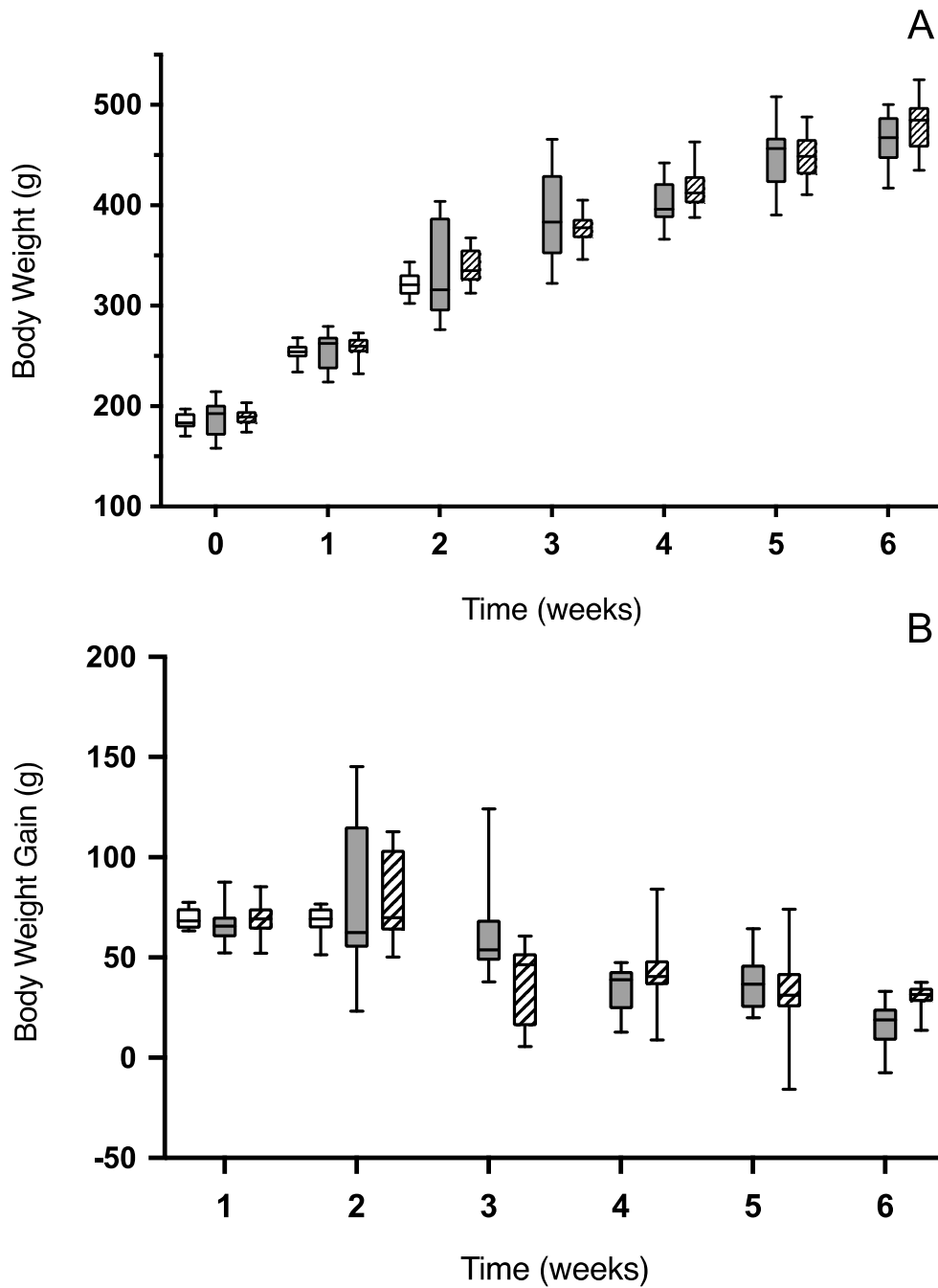


Figure S1. Body weight and body weight gain Body weight (A) and weekly body weight gain (B) was similar in animals of group 1 (empty boxes), group 2 on standard-Ca-diet (gray boxes) and group 3 on low-Ca-diet (hatched boxes) throughout the study.

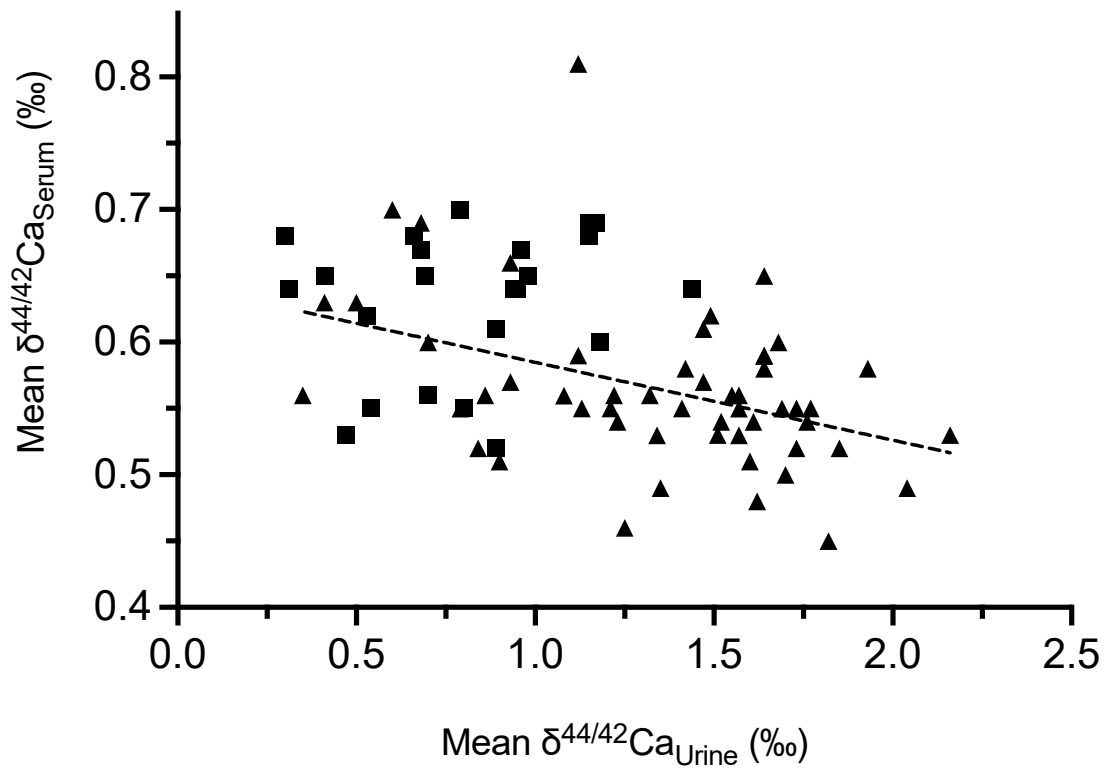


Figure S2. Mean $\delta^{44/42}\text{Ca}_{\text{Urine}}$ and $\delta^{44/42}\text{Ca}_{\text{Serum}}$ in rats on low and standard-Ca-diet Two weeks means of $\delta^{44/42}\text{Ca}_{\text{Urine}}$ in rats on standard-diet (triangles) and low-Ca-diet (squares) are shown relative to the respective mean $\delta^{44/42}\text{Ca}_{\text{Serum}}$. In animals on standard-Ca-diet $\delta^{44/42}\text{Ca}_{\text{Urine}}$ inversely correlated with $\delta^{44/42}\text{Ca}_{\text{Serum}}$ ($r=-0.41$, $p=0.003$; $n=53$) but not in animals on low-Ca-diet ($r=0.18$, $p=0.39$; $n=23$).