

SUPPLEMENTARY DATA

for

The role of renal RhCG in metabolic responses to dietary protein

Lisa Bounoure*, Davide Ruffoni‡, Ralph Müller‡, Gisela Anna Kuhn‡, Soline Bourgeois*, Olivier Devuyst*, Carsten A. Wagner*

*Institute of Physiology and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland, ‡Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

Correspondence

Carsten A. Wagner
Institute of Physiology
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich
Switzerland
+41(0)44 635 50 23
+41 (0)44 635 68 14
wagnerca@access.uzh.ch

Supplementary table 1

Body weight, food intake and urinary values of *Rhcg^{+/+}*, *Rhcg^{+/-}*, and *Rhcg^{-/-}* mice during 9 days high soy (HS) protein loading (n = 6 mice for each group). # $P \leq 0.05$ significantly different from same genotype under control conditions

Supplementary table 2

Blood values of *Rhcg^{+/+}*, *Rhcg^{+/-}*, and *Rhcg^{-/-}* mice on normal diet and during the high casein (HC) protein loading (n = 8 mice for each group). ND not determined, # $P \leq 0.05$ significantly different from same genotype under control conditions, * $P \leq 0.05$ significantly different from *Rhcg^{+/+}* mice under same treatment conditions, ** $P \leq 0.01$ significantly different from *Rhcg^{+/+}* mice under same treatment conditions

Supplementary table 3

Blood values of *Rhcg^{+/+}*, *Rhcg^{+/-}*, and *Rhcg^{-/-}* mice on normal diet and during the high soy (HS) protein loading (n = 6 mice for each group). ND not determined, # $P \leq 0.05$ significantly different from same genotype under control conditions

Supplementary table 4

Urinary values of *Rhcg^{+/+}*, *Rhcg^{+/-}*, and *Rhcg^{-/-}* mice during 9 days high casein (HC) protein loading (n = 8 mice for each group). ND not determined, # $P \leq 0.05$ significantly different from same genotype under control conditions, * $P \leq 0.05$ significantly different from *Rhcg^{+/+}* mice under same treatment conditions, ** $P \leq 0.01$ significantly different from *Rhcg^{+/+}* mice under same treatment conditions

Supplementary table 5

Standard morphometric parameters of *Rhcg^{+/+}* and *Rhcg^{-/-}* mice during 9 days high casein (HC) protein loading (n = 5 mice for each group).

Supplementary figure 1. Nine days high soy diet does not acid load of *Rhcg*^{+/+}, ⁺⁻ and ^{-/-} mice

Blood and urine data were collected in *Rhcg*^{+/+}, ⁺⁻ and ^{-/-} mice treated for 9 days with high soy (HS) diet. HS did not induce acid load as indicated by blood pH (A) and bicarbonate (B) and urinary titratable acidity (C) or NH₄⁺ excretion (D). Values are mean ± SEM (n= 6 mice) *p ≤ 0.05 (*Rhcg*^{-/-} vs *Rhcg*^{+/+}), # P ≤ 0.05 significantly different from same genotype under control conditions (day 0)

Supplementary figure 2. *Rhcg* mRNA expression is altered by HC diet while *Rhbg* mRNA level is unchanged.

Rhcg and *Rhbg* mRNA expression was measured by RT-qPCR in kidneys from *Rhcg*^{+/+} and ^{-/-} mice during 0, 4 and 9 days HC diet. Four days HC diet induced a transient increase of *Rhcg* mRNA expression (A). mRNA levels of *Rhbg* remained unchanged in *Rhcg*^{+/+} and ^{-/-} after 0, 4 and 9 days HC. Values are mean ± SEM (n= 6 mice), # P ≤ 0.05 significantly different from same genotype under control conditions (day 0)

Supplementary figure 3. mRNA and protein expression of PEPCK and NHE3 proximal tubule proteins are unchanged by HC diet

Immunoblotting of *Rhcg*^{+/+} kidney protein extracts collected during HC diet did not show any changes in PEPCK (A) or NHE3 (B) expression in response to the treatment. PEPCK (C) and NHE3 (D) mRNA levels assessed by RT-qPCR did not display either differences comparing *Rhcg*^{+/+}, ⁺⁻ and ^{-/-} after 9 days HC. Similarly, PEPCK and NHE3 protein levels were identical comparing all 3 groups of mice 4 or 9 days-HC diet fed. Values are mean ± SEM (n= 8 mice), p ≥ 0.05

Supplementary figure 4. High soy protein diet does not alter NKCC2 and AQP2 protein abundance in *Rhcg*^{+/+} mice.

The expression of NKCC2 (A) and AQP2 (B) measured by immunoblotting in kidneys from *Rhcg*^{+/+} mice and remained similar between day 0 and 9 of high soy diet. Values are mean ± SEM (n= 8 mice), p ≥ 0.05

Supplementary table 1. Body weight, food intake and urinary values of *Rhcg*^{+/+}, *Rhcg*⁺⁻, and *Rhcg*^{-/-} mice during 9 days high soy (HS) protein loading

	Basal status			2 days HS diet			4 days HS diet			9 days HS diet		
	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ⁺⁻ (n=6)	<i>Rhcg</i> ^{-/-} (n=6)	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ⁺⁻ (n=6)	<i>Rhcg</i> ^{-/-} (n=6)	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ⁺⁻ (n=6)	<i>Rhcg</i> ^{-/-} (n=6)	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ⁺⁻ (n=6)	<i>Rhcg</i> ^{-/-} (n=6)
Body weight (g)	28.5 ± 1.6	26.0 ± 1.1	27.2 ± 1.4	27.6 ± 1.5	25.2 ± 1.1	25.6 ± 1.3	26.8 ± 1.3	25.0 ± 1.1	25.6 ± 1.3	25.8 ± 0.9	25.0 ± 1.2	25.2 ± 1.1
Food intake (g/24hrs/body weight)	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Water intake (g/24hrs/body weight)	0.15 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.22 ± 0.03 [#]	0.26 ± 0.02 [#]	0.23 ± 0.02 [#]	0.23 ± 0.01 [#]	0.23 ± 0.02 [#]	0.25 ± 0.03 [#]	0.27 ± 0.03 [#]	0.28 ± 0.02 [#]	0.27 ± 0.03 [#]
Urine values												
Volume (ml/24 h)	1.84 ± 0.22	1.83 ± 0.27	1.79 ± 0.20	2.95 ± 0.38 [#]	2.87 ± 0.35 [#]	3.37 ± 0.56 [#]	2.58 ± 0.18 [#]	2.03 ± 0.26 [#]	2.50 ± 0.39 [#]	3.24 ± 0.33 [#]	2.53 ± 0.35 [#]	3.36 ± 0.30 [#]
Creatinine excretion (μmol/24h)	10.18 ± 0.53	11.52 ± 1.18	10.70 ± 1.57	7.92 ± 1.47 [#]	7.15 ± 0.77 [#]	7.51 ± 0.50 [#]	5.98 ± 0.71 [#]	4.76 ± 0.59 [#]	5.34 ± 0.49 [#]	6.92 ± 1.19 [#]	5.71 ± 1.12 [#]	7.11 ± 1.08 [#]
Urinary pH	5.91 ± 0.08	5.63 ± 0.07	5.70 ± 0.11	6.83 ± 0.18 [#]	7.06 ± 0.23 [#]	6.95 ± 0.17 [#]	7.09 ± 0.14 [#]	7.26 ± 0.09 [#]	7.29 ± 0.11 [#]	7.11 ± 0.27 [#]	7.15 ± 0.13 [#]	6.99 ± 0.17 [#]
NH ₄ ⁺ /24h (μmol/24h)	46.9 ± 4.0	53.1 ± 4.3	46.2 ± 3.9	14.8 ± 1.5 [#]	12.9 ± 1.2 [#]	13.7 ± 2.0 [#]	10.9 ± 2.9 [#]	12.0 ± 1.6 [#]	12.9 ± 1.2 [#]	17.1 ± 1.8 [#]	15.3 ± 1.7 [#]	15.1 ± 1.9 [#]
TA/24h (μmol/24h)	211.1 ± 11.5	182.8 ± 11.8	197.3 ± 12.1	19.1 ± 1.8 [#]	20.7 ± 1.9 [#]	23.3 ± 1.5 [#]	27.1 ± 1.7 [#]	23.22 ± 1.8 [#]	24.1 ± 1.9 [#]	23.3 ± 1.6 [#]	28.4 ± 1.6 [#]	25.2 ± 1.5 [#]
PO ₄ ²⁻ /Crea (μmol/24h)	35.1 ± 2.8	38.0 ± 2.4	39.8 ± 3.6	16.8 ± 2.3 [#]	18.2 ± 3.3 [#]	19.9 ± 3.1 [#]	22.3 ± 2.8 [#]	19.5 ± 3.2 [#]	20.6 ± 3.9	19.2 ± 2.3 [#]	23.0 ± 4.7 [#]	18.5 ± 3.6 [#]
Urea/24h (μmol/24h)	2.31 ± 0.52	2.64 ± 0.37	2.86 ± 0.78	6.90 ± 0.35 [#]	7.33 ± 0.98 [#]	7.02 ± 0.78 [#]	6.18 ± 0.65 [#]	7.51 ± 0.87 [#]	6.97 ± 0.83 [#]	8.21 ± 0.62 [#]	7.69 ± 0.47 [#]	8.02 ± 0.39 [#]
Ca ²⁺ /24h (μmol/24h)	2.16 ± 0.02	2.21 ± 0.03	2.33 ± 0.07	1.99 ± 0.05	2.09 ± 0.07	2.01 ± 0.02	2.14 ± 0.07	2.10 ± 0.03	2.16 ± 0.04	1.87 ± 0.06	1.98 ± 0.08	2.03 ± 0.07
Na ⁺ /Crea ((μmol/24h)	70.5 ± 4.5	75.5 ± 3.9	71.8 ± 4.5	150.5 ± 13.2 [#]	162.0 ± 17.0 [#]	150.7 ± 18.3 [#]	182.9 ± 19.3 [#]	191.8 ± 19.5 [#]	205.8 ± 19.7 [#]	214.1 ± 15.2 [#]	201.0 ± 13.0 [#]	198.9 ± 11.3 [#]
Cl ⁻ /Crea (mmol/24h)	0.31 ± 0.02	0.35 ± 0.03	0.33 ± 0.04	0.45 ± 0.05 [#]	0.48 ± 1.00 [#]	0.51 ± 0.09 [#]	0.51 ± 0.04 [#]	0.55 ± 0.04 [#]	0.61 ± 0.09 [#]	0.64 ± 0.05 [#]	0.49 ± 0.07 [#]	0.57 ± 0.08 [#]
K ⁺ /Crea (mmol/24h)	0.28 ± 0.04	0.37 ± 0.06	0.31 ± 0.08	0.44 ± 0.03 [#]	0.54 ± 0.05 [#]	0.58 ± 0.02 [#]	0.56 ± 1.0 [#]	0.49 ± 0.08 [#]	0.52 ± 0.09 [#]	0.62 ± 0.05 [#]	0.54 ± 0.03 [#]	0.59 ± 0.09 [#]
SO ₄ ²⁻ /24 h (μmol/24 h)	0.26 ± 0.04 n=6	0.21 ± 0.02 n=5	0.24.0 ± 0.07 n=6	ND	ND	ND	ND	ND	ND	0.30 ± 0.03 n=6	0.27 ± 0.06 n=5	0.28 ± 0.03 n=6

Supplementary Table 2. Blood values of *Rhcg*^{+/+}, *Rhcg*^{+/-}, and *Rhcg*^{-/-} mice on normal diet and during the high casein (HC) protein loading

	Basal status			2 days HC diet			4 days HC diet		9 days HC diet		
	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)	<i>Rhcg</i> ^{+/+} (n=5)	<i>Rhcg</i> ^{-/-} (n=5)	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)
pH	7.35 ± 0.01	7.33 ± 0.01	7.34 ± 0.01	7.26 ± 0.03 [#]	7.27 ± 0.01 [#]	7.28 ± 0.03 [#]	7.36 ± 0.01	7.34 ± 0.01	7.37 ± 0.02	7.35 ± 0.01	7.34 ± 0.02
pCO ₂ (mmHg)	37.4 ± 1.1	39.5 ± 1.0	37.5 ± 0.8	39.1 ± 1.4	38.4 ± 1.1	36.9 ± 1.1	37.1 ± 1.1	39.5 ± 1.8	40.2 ± 1.1	38.5 ± 1.2	38.9 ± 1.4
HCO ₃ ⁻ (mM)	20.1 ± 0.4	19.0 ± 0.3	19.7 ± 0.4	17.2 ± 1.4 [#]	16.9 ± 0.5 [#]	15.5 ± 1.5 [#]	22.2 ± 0.4	20.8 ± 0.9	22.5 ± 0.8	20.7 ± 0.7	20.1 ± 1.1
Hct (%)	49.9 ± 0.2	49.4 ± 0.6	50.2 ± 1.0	51.1 ± 0.7	50.7 ± 0.7	52.6 ± 0.7	49.6 ± 0.2	50.6 ± 0.6	53.5 ± 0.8 [#]	52.2 ± 0.7 [#]	55.8 ± 0.6 [#]
Na ⁺ (mM)	146.5 ± 0.5	146.9 ± 0.4	145.5 ± 0.4	147.5 ± 0.4	148.7 ± 0.4	147.6 ± 0.7	144.8 ± 0.6	145.4 ± 0.5	147.8 ± 0.5	148.5 ± 0.5	146.5 ± 0.4
Cl ⁻ (mM)	112.9 ± 0.4	114.1 ± 0.6	111.9 ± 0.5	117.7 ± 1.0 [#]	118.3 ± 0.9 [#]	121.4 ± 1.2 [#]	110.4 ± 1.3	112.0 ± 0.8	116.8 ± 1.2 [#]	116.3 ± 1.0 [#]	119 ± 1.7 [#]
Ionized Ca ²⁺ (mM)	1.24 ± 0.006	1.26 ± 0.009	1.24 ± 0.002	1.33 ± 0.005 [#]	1.30 ± 0.004 [#]	1.39 ± 0.01*** [#]	1.25 ± 0.008	1.30 ± 0.009** [#]	1.28 ± 0.009	1.29 ± 0.001	1.25 ± 0.006
K ⁺ (mM)	4.6 ± 1.2	4.4 ± 1.1	4.5 ± 1.1	4.8 ± 0.2	4.6 ± 0.2	4.1 ± 0.1	4.9 ± 0.2	4.7 ± 0.7	4.6 ± 0.1	4.6 ± 0.2	4.3 ± 0.2
Glucose (mM)	10.6 ± 0.6	10.7 ± 0.5	11.7 ± 0.5	8.0 ± 1.1 [#]	8.9 ± 0.5 [#]	9.4 ± 0.7 [#]	11.9 ± 0.4	12.1 ± 0.5	10.0 ± 0.9	10.2 ± 0.6	12.2 ± 0.9
Lactate (mM)	3.6 ± 0.3	3.6 ± 0.2	3.9 ± 0.7	3.8 ± 0.4	3.3 ± 0.3	3.3 ± 0.3	3.5 ± 0.2	3.0 ± 0.4	2.4 ± 0.2 [#]	2.6 ± 0.2 [#]	2.9 ± 0.3 [#]
Osteocalcin (ng/mL)	152.5 ± 20.4	ND	135.7 ± 20.0	ND	ND	ND	144.8 ± 10.0	108.1 ± 18.7	132.1 ± 15.1	ND	113.9 ± 10.4

Supplementary table 3. Urinary values of *Rhcg*^{+/+}, *Rhcg*^{+/-}, and *Rhcg*^{-/-} mice during 9 days high casein (HC) protein loading

	Basal status			2 days HC diet			4 days HC diet			9 days HC diet		
	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)	<i>Rhcg</i> ^{+/+} (n=8)	<i>Rhcg</i> ^{+/-} (n=8)	<i>Rhcg</i> ^{-/-} (n=8)
Creatinine excretion ($\mu\text{mol}/24\text{h}$)	6.53 \pm 0.38	6.81 \pm 0.99	6.89 \pm 0.85	6.45 \pm 0.70	6.96 \pm 0.73	6.59 \pm 0.41	6.04 \pm 0.60	6.56 \pm 0.21	6.22 \pm 0.81	6.57 \pm 0.52	6.48 \pm 0.33	6.85 \pm 0.72
$\text{PO}_4^{2-}/\text{Crea}$ ($\mu\text{mol}/24\text{h}$)	48.1 \pm 2.5	45.7 \pm 2.3	42.6 \pm 2.6	97.6 \pm 3.2#	99.7 \pm 3.0#	98.7 \pm 4.1#	112.6 \pm 3.8#	118.9 \pm 3.9#	119.2 \pm 4.3#	145.1 \pm 3.9#	137.5 \pm 4.5#	158.8 \pm 4.3#
Urea/Urine volume ($\mu\text{mol}/24\text{h}$)	2.40 \pm 0.17	2.60 \pm 0.28	2.81 \pm 0.26	7.79 \pm 0.75#	7.81 \pm 0.61#	7.89 \pm 0.63#	6.37 \pm 0.65#	8.27 \pm 0.74#	8.12 \pm 0.99#	6.89 \pm 0.58#	9.48 \pm 0.67#	10.84 \pm 0.40*#
Na^+/Crea ($\mu\text{mol}/24\text{h}$)	132.3 \pm 4.7	128.0 \pm 3.9	133.6 \pm 7.4	156.4 \pm 7.5	166.5 \pm 8.9	172.5 \pm 10.2	168.3 \pm 9.0	175.9 \pm 3.8	181.7 \pm 9.1	175.6 \pm 6.1	177.9 \pm 9.2	189.8 \pm 13.5
Cl^-/Crea (mmol/24h)	0.31 \pm 0.01	0.40 \pm 0.03	0.36 \pm 0.06	0.42 \pm 0.05	0.37 \pm 0.07	0.46 \pm 0.09	0.45 \pm 0.06	0.48 \pm 0.04	0.53 \pm 0.01	0.54 \pm 0.1	0.50 \pm 0.05	0.59 \pm 0.04
K^+/Crea (mmol/24h)	0.55 \pm 0.03	0.62 \pm 0.05	0.59 \pm 0.04	0.79 \pm 0.08	0.70 \pm 1.06	0.83 \pm 1.03	0.84 \pm 1.09	0.97 \pm 0.09	1.13 \pm 0.11	1.10 \pm 0.08	1.15 \pm 1.02	1.24 \pm 1.09
$\text{SO}_4^{2-}/\text{Urine volume}$ ($\mu\text{mol}/24\text{h}$)	0.22 \pm 0.9	0.18 \pm 0.2	0.23 \pm 0.4	0.43 \pm 0.8#	0.49 \pm 0.5#	0.53 \pm 0.1#	0.51 \pm 0.6#	0.53 \pm 0.5#	0.47 \pm 0.7#	0.52 \pm 0.8#	0.58 \pm 0.9#	0.56 \pm 0.7#

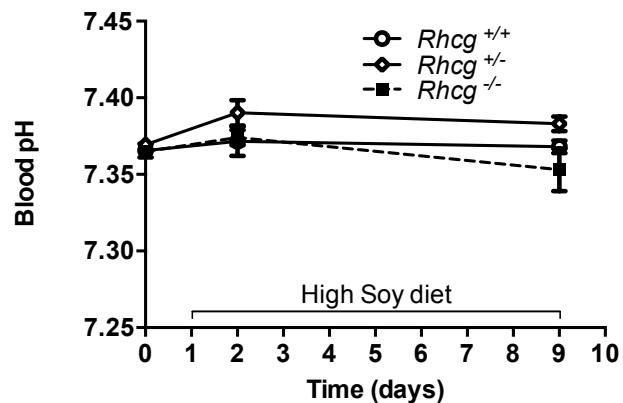
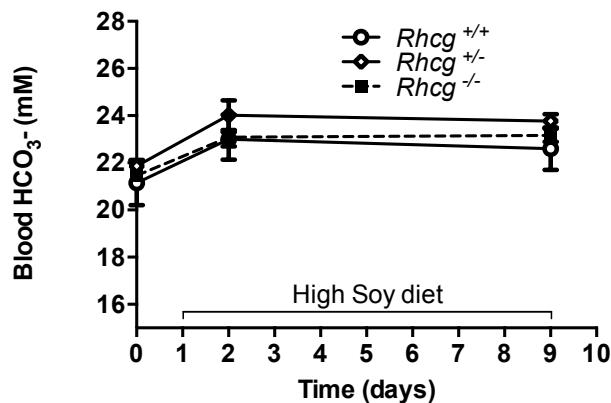
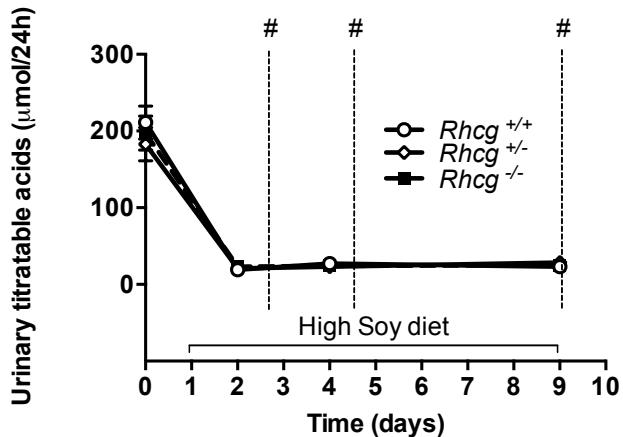
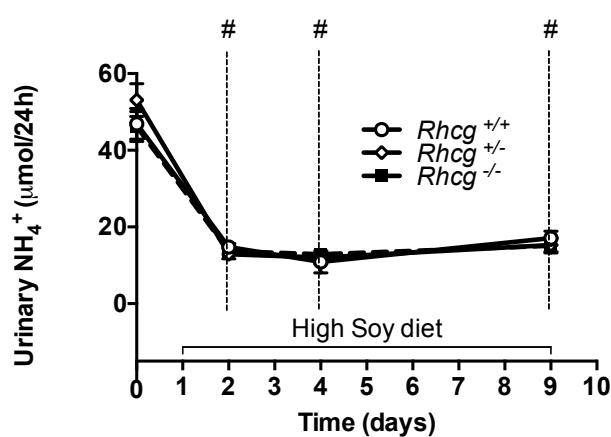
Supplementary table 4. Blood values of *Rhcg*^{+/+}, *Rhcg*^{+/-}, and *Rhcg*^{-/-} mice on normal diet and during the high soy (HS) protein loading.

	Basal status			2 days HS diet			9 days HS diet		
	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ^{+/-} (n=6)	<i>Rhcg</i> ^{-/-} (n=6)	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ^{+/-} (n=6)	<i>Rhcg</i> ^{-/-} (n=6)	<i>Rhcg</i> ^{+/+} (n=6)	<i>Rhcg</i> ^{+/-} (n=6)	<i>Rhcg</i> ^{-/-} (n=6)
pH	7.36 ± 0.004	7.37 ± 0.004	7.36 ± 0.003	7.37 ± 0.02	7.39 ± 0.02	7.37 ± 0.01	7.37 ± 0.02	7.38 ± 0.02	7.35 ± 0.01
pCO ₂ (mmHg)	38.8 ± 0.8	38.9 ± 0.2	38.2 ± 0.5	38.1 ± 1.1	39.2 ± 0.8	39.5 ± 1.9	39.1 ± 1.7	39.7 ± 1.5	39.9 ± 1.3
HCO ₃ ⁻ (mM)	21.1 ± 0.9	21.9 ± 0.2	21.5 ± 0.2	23.1 ± 1.4	24 ± 0.8	23.1 ± 0.9	22.6 ± 0.8	23.8 ± 0.3	23.2 ± 0.3
Hct (%)	49.4 ± 0.2	49.4 ± 0.4	49.0 ± 0.5	49.2 ± 0.7	49.5 ± 0.8	48.8 ± 1.1	49.3 ± 0.6	49.2 ± 0.8	49.1 ± 0.9
Na ⁺ (mM)	147.5 ± 0.5	149.3 ± 0.8	149.8 ± 0.4	148 ± 0.4	148.4 ± 0.5	148.7 ± 0.9	147.5 ± 0.5	147.2 ± 0.5	148.3 ± 0.6
Cl ⁻ (mM)	115.0 ± 0.1	114.3 ± 0.4	115.1 ± 0.6	114.0 ± 0.9	113.6 ± 0.4	113.8 ± 2.0	111.1 ± 0.1 [#]	112.0 ± 0.5 [#]	111.5 ± 0.9 [#]
Ionized Ca ²⁺ (mM)	1.30 ± 0.005	1.27 ± 0.008	1.30 ± 0.005	1.29 ± 0.001	1.26 ± 0.001	1.25 ± 0.002	1.27 ± 0.04	1.28 ± 0.01	1.23 ± 0.01 [#]
K ⁺ (mM)	5.2 ± 0.3	5.0 ± 0.2	5.2 ± 0.1	5.1 ± 0.5	5.1 ± 0.2	5.3 ± 0.3	5.3 ± 0.2	5.2 ± 0.3	5.2 ± 0.2
Glucose (mM)	12.1 ± 0.2	12.3 ± 0.4	12.0 ± 0.3	11.8 ± 0.4	11.2 ± 1.0	12.7 ± 1.2	12.0 ± 0.9	11.9 ± 1.0	11.8 ± 1.5
Lactate (mM)	4.7 ± 0.1	4.3 ± 0.3	4.4 ± 0.5	3.5 ± 1.3	3.7 ± 0.3	4.1 ± 0.7	3.4 ± 0.6	3.5 ± 0.2	3.3 ± 0.7

Supplementary table 5. Standard morphometric parameters from *Rhcg*^{+/+} and *Rhcg*^{-/-} during 9 days high casein protein loading

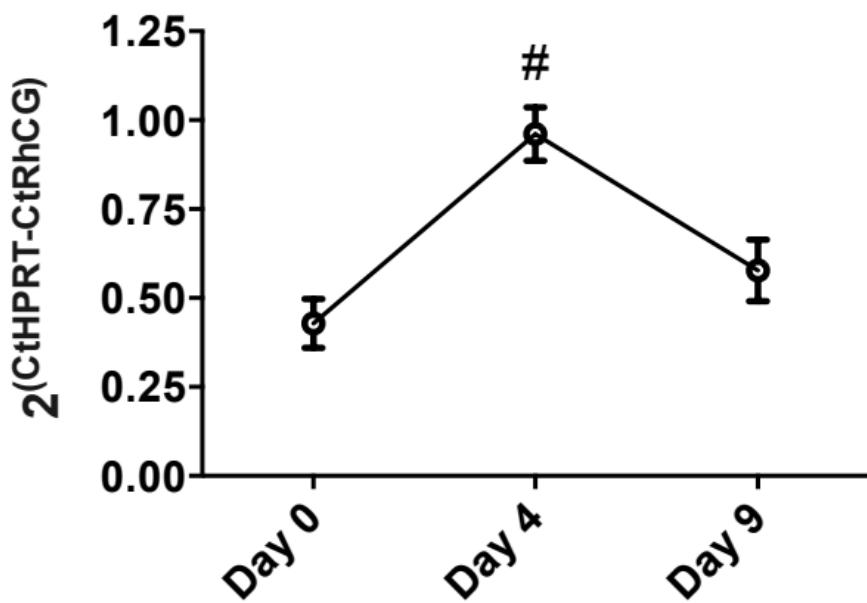
	<i>Rhcg</i> ^{+/+}	<i>Rhcg</i> ^{-/-}
	(n=5)	(n=5)
Trabecular compartment		
Bone volume fraction (%)	16.32 ± 1.56	15.27 ± 0.78
Trabecular number (1/mm)	3.79 ± 0.47	3.38 ± 0.31
Trabecular thickness (mm)	0.060 ± 0.003	0.062 ± 0.003
Trabecular separation (mm)	0.277 ± 0.098	0.306 ± 0.037
Cortical compartment		
Total cross-sectional area inside the periosteal envelope (mm ²)	1.000 ± 0.046	1.076 ± 0.046
Cortical bone area (mm ²)	0.954 ± 0.045	1.029 ± 0.045
Cortical area fraction (mm ³)	0.954 ± 0.001	0.956 ± 0.001
Average cortical thickness (mm)	0.237 ± 0.007	0.241 ± 0.008
Full bone		
Apparent volume density (%)	57.44 ± 0.96	57.66 ± 0.99

Supplementary figure 1

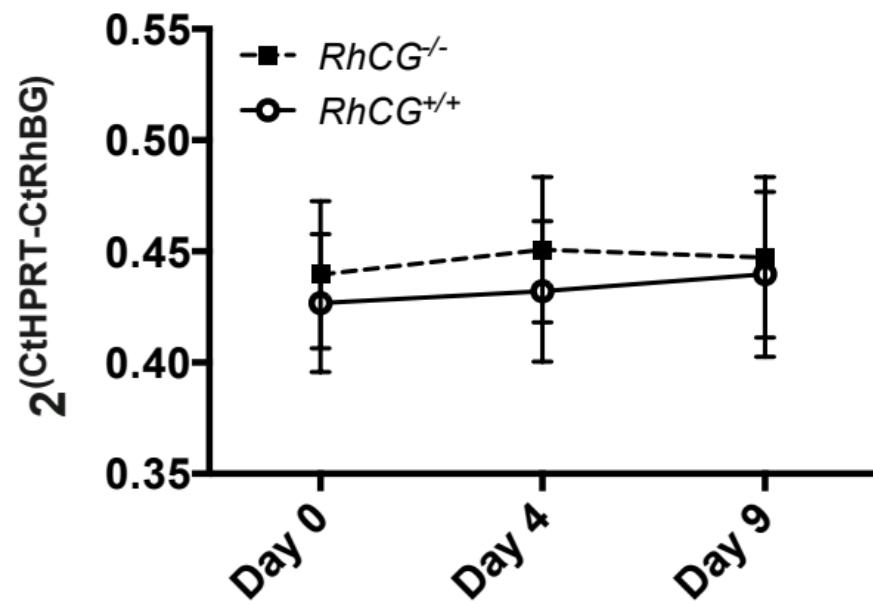
A**B****C****D**

Supplementary figure 2

A

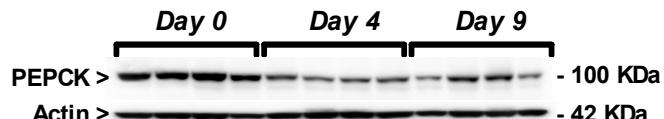


B



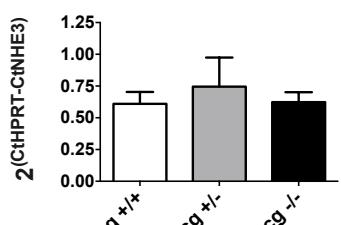
Supplementary figure 3

A

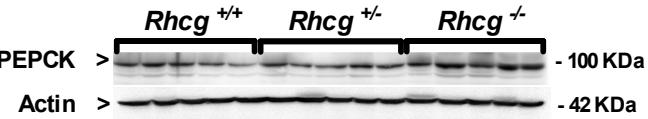


C

High Casein diet, *Rhcg*^{+/+}

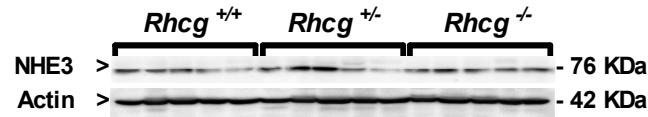


E



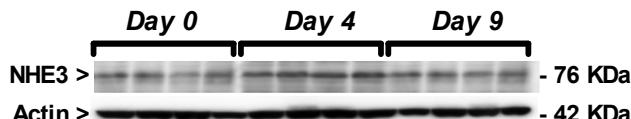
High Casein diet, 4 days

G



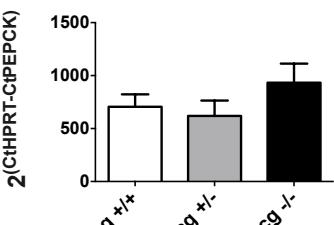
High Casein diet, 4 days

B



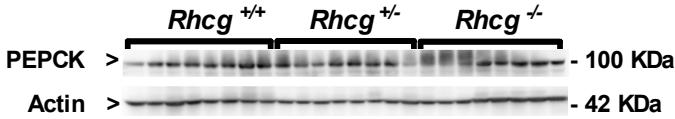
D

High Casein diet, *Rhcg*^{+/+}



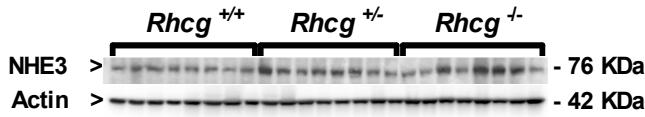
High Casein diet, 9 days

F



High Casein diet, 9 days

H



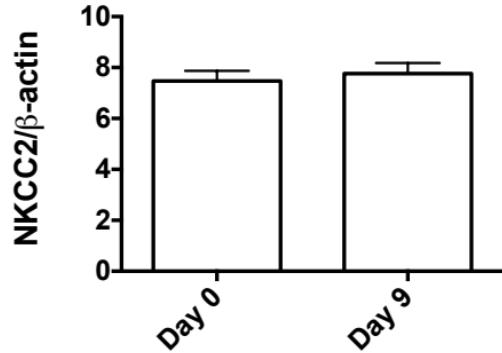
High Casein diet, 9 days

Supplementary figure 4

A

High Soy diet, $Rhcg^{+/+}$

Day 0 Day 9



B

High Soy diet, $Rhcg^{+/+}$

Day 0 Day 9

