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Supplemental Data

Short Article

Increased Energy Expenditure, Dietary Fat Wasting, and Resistance to Diet-Induced Obesity in Mice Lacking Renin

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Table S1. Body Composition, Tissue Weights, and Body Length of 10-Month-Old Mice

	regular chow		high-fat diet	
	WT	*Ren1c-/-*	WT	*Ren1c-/-*
body weight (g)	36.4 ± 1.3	29.5 ± 0.6[c]	51.7 ± 3.2[a]	29.7 ± 0.5[d]
body length (cm)	9.8 ± 0.1	9.5 ± 0.2	9.7 ± 0.2	9.5 ± 0.3
lean tissue (g)	24.6 ± 0.9	25.7 ± 1.2	32.1 ± 0.8[a]	25.4 ± 0.6[d]
body fat (g)	10.7 ± 1.2	5.1 ± 0.7[c]	20.8 ± 0.8[a]	5.5 ± 0.5[d]
% body fat	29.3 ± 2.8	17.2 ± 2.4[c]	40.2 ± 0.8[a]	19.1 ± 1.2[d]
IWAT (g)	1.03 ± 0.18	0.23 ± 0.10[c]	1.48 ± 0.19[a]	0.29 ± 0.03[d]
GWAT (g)	2.70 ± 0.33	0.56 ± 0.29[c]	3.95 ± 0.21[a]	0.98 ± 0.01[d]
RWAT (g)	0.82 ± 0.11	0.17 ± 0.11[c]	1.43 ± 0.29[a]	0.28 ± 0.03[d]
BAT (g)	0.30 ± 0.02	0.14 ± 0.02[c]	0.32 ± 0.05	0.10 ± 0.01[d]
heart (g)	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.02	0.14 ± 0.02
liver (g)	1.39 ± 0.13	1.28 ± 0.05	3.80 ± 0.60[a]	1.08 ± 0.02[d]
fat (g/g liver)	0.16 ± 0.01	0.15 ± 0.01	0.94 ± 0.07[a]	0.44 ± 0.07[bd]
fat (g/g soleus)	ND	ND	0.33 ± 0.13	0.32 ± 0.17
pancreas (g)	ND	ND	0.23 ± 0.02	0.22 ± 0.02
small intestine length (cm)	ND	ND	34.0 ± 0.8	33.6 ± 1.0

Body length: nose to rump length, IWAT, inguinal white adipose tissue; GWAT, gonadal WAT; RWAT, retroperitoneal WAT; BAT, interscapular brown adipose tissue. Body fat was measured by dual-energy X-ray absorptiometry. [a, c]$p < 0.05$ vs. WT on regular chow; [b]$p < 0.05$ vs. *Ren1c-/-* on regular chow; [d]$p < 0.05$ vs. WT on a high-fat diet. Two month old males were fed either regular chow or a high-fat diet for 8 months. n = 6~10. ND, not determined.

Table S2. Metabolic Studies by Oxymax

	WT	*Ren1c-/-*	p value
body weight (g)	26.1 ± 1.4	21.5 ± 0.9	0.016
lean body mass (g)	18.7 ± 0.6	18.0 ± 0.7	0.482
fat body mass (g)	6.3 ± 0.7	2.3 ± 0.2	0.001
total O2 consumption (ml/kg/h)	5256 ± 290	6868 ± 333	0.002
resting O2 consumption (ml/kg/h)	3758 ± 293	5522 ± 313	0.001
total heat generation (kcal/kg/d)	629 ± 35	801 ± 39	0.005
resting heat generation (kcal/kg/d)	448 ± 35	634 ± 34	0.002
total O2 consumption (ml/kg lean/h)	7139 ± 268	8247 ± 339	0.022
resting O2 consumption (ml/kg lean/h)	5105 ± 303	6609 ± 345	0.006
total heat generation (kcal/kg lean/d)	854 ± 32	961 ± 39	0.048
resting heat generation (kcal/kg lean/d)	608 ± 36	758 ± 36	0.011
respiratory quotient (RQ)	0.95 ± 0.03	0.85 ± 0.02	0.020
horizontal activity	358 ± 38	372 ± 49	0.826
ambulation	194 ± 25	202 ± 31	0.856
vertical activity	35 ± 11	33 ± 12	0.896

Animals were 3~5 months old and fed a high-fat diet for 2 weeks before the measurements. Lean and fat body mass were measured using MRI. n = 9 each.

Table S3. Gene Expression of Uncoupling Proteins

	WT	*Ren1c-/-*	p value
UCP1, brown adipose tissue	100 ± 25	1620 ± 320	0.002
UCP2, gonadal white adipose tissue	100 ± 10	153 ± 15	0.019
UCP3, brown adipose tissue	100 ± 11	135 ± 11	0.043
UCP3, skeletal muscle	100 ± 32	435 ± 128	0.046

Animals were 3~5-month-old males fed a high-fat diet for 4 weeks. n = 6~8 each.

Table S4. Catecholamines

	WT	*Ren1c-/-*
NE-plasma, ng/ml	1.2 ± 0.4	1.0 ± 0.2
Epi-plasma, ng/ml	1.2 ± 0.3	1.1 ± 0.3
NE- Heart, ng/heart/hr	23.0 ± 1.9	21.0 ± 1.8
NE- GWAT, ng/hr/mgRNA	124 ± 17	136 ± 12
NE- IWAT, ng/hr/mgRNA	139 ± 20	126 ± 7
NE- BAT, ng/hr/mgRNA	1540 ± 160	1890 ± 180

Synthesis of norepinephrine (NE) and epinephrine (Epi) was calculated as described in materials and methods. The amount of NE in adipose tissues was normalized by the amount of total RNA, which is proportional to the number of cells (O'Brien et al., 1996). n = 8 each. Animals were 4~6-month-old males fed a high-fat diet for 4 weeks.

O'Brien, S. N., Mantzke, K. A., Kilgore, M. W., and Price, T. M. (1996). Relationship between adipose stromal-vascular cells and adipocytes in human adipose tissue. Anal Quant Cytol Histol *18*, 137-143.

Table S5. Plasma Levels of Thyroid Hormones

	WT	*Ren1c*-/-	p value
T3 (ng/dl)	113.8 ± 20.0	67.8 ± 6.3	0.05
T4 (μg/dl)	16.6 ± 3.0	7.8 ± 2.4	0.03

Animals were 3~5-month-old males fed a high-fat diet for 4 weeks. n = 8 each.

Table S6. Bile Acid Contents in the Liver and Intestine

	WT	*Ren1c*-/-
liver (μmol/mouse)	3.5 ± 0.6	4.0 ± 0.8
intestine (μmol/mouse)	11.3 ± 1.0	11.5 ± 0.9

Animals were 3~5-month-old males fed a high-fat diet for 4 weeks. n = 8 each.

Table S7. Expression of Adipogenic Genes in Gonadal WAT

	WT	*Ren1c*-/-
cyclin D1	100 ± 22	157 ± 37
C/EBPβ	100 ± 26	101 ± 25
C/EBPα	100 ± 11	85 ± 18
PPARγ	100 ± 12	108 ± 15
SREBP1a	100 ± 12	108 ± 15
SREBP1c	100 ± 13	126 ± 17
DGAT2	100 ± 5	98 ± 4

Values are expressed as % WT. Animals were 3~5-month-old males fed a high-fat diet for 4 weeks. n = 6~8.

Table S8. Plasma Biochemistry of Insulin Sensitivity and Lipid Metabolism

	Regular Chow		High-Fat Diet	
	WT	*Ren1c*-/-	WT	*Ren1c*-/-
glucose (mg/dl)	148.6 ± 13.1	134.2 ± 8.7	174.5 ± 15.0	121.5 ± 17.4[d]
insulin (ng/ml)	1.1 ± 0.4	0.7 ± 0.3	3.8 ± 0.8[a]	0.5 ± 0.2[d]
triglyceride (mg/dl)	79.7 ± 1.8	84.3 ± 2.8	97.3 ± 4.8[a]	89.1 ± 4.7
total cholesterol (mg/dl)	26.3 ± 1.9	28.8 ± 2.3	58.3 ± 2.8[a]	57.6 ± 2.1[b]
free fatty acid (mM)	0.87 ± 0.05	0.72 ± 0.02	0.50 ± 0.03[a]	0.43 ± 0.03[b]
adiponectin (μg/ml)	20.7 ± 1.3	29.5 ± 0.8[c]	26.4 ± 3.4	75.4 ± 3.9[bd]
leptin (ng/ml)	35.1 ± 5.1	24.3 ± 1.8	107.2 ± 12.5[a]	36.4 ± 4.5[bd]

Animals are the same as those in Table 1, and were 10-month-old males fed either regular chow or a high-fat diet for 8 months. Blood was drawn after 4 hrs of fasting. n = 6~10. [a, c]$p < 0.05$ vs. WT on regular chow; [b]$p < 0.05$ vs. *Ren1c*-/- on regular chow; [d]$p < 0.05$ vs. WT on a high-fat diet.

Table S9. Primers and Probes for qRT-PCR

adiponectin:	5'-ATC CTG GCC ACA ATG GCA CA-3'
	5'-CAA GAA GAC CTG CAT CTC CT-3'
	5'-FAM-CCG TGA TGG CAG AGA TGG CAC TCC T-TAMRA-3'
colipase	5'-ACA CAC AAG GCC ATG GAG AA-3'
	5'-TCA CAG GGA CAC CGG TAG T-3'
	5'-FAM-CGA GTG CTC CCC AAA GAC CCT CTA-TAMRA-3'
cyclin D1:	5'-CTA CAC TGA CAA CTC TAT CCG-3'
	5'-TGA AAT CGT GGG GAG TCA TG-3'
	5'-FAM- TGC AAA TGG AAC TGC TTC TGG TGA ACA-TAMRA-3'
C/EBPα:	5'-CAG CAA CGA GTA CCG GGT A-3'
	5'-TGC GTC TCC ACG TTG CGT T-3'
	5'-FAM- CAA CAA CAT CGC G/TGT GCG CAA GAG CC-TAMRA-3'
C/EBPβ:	5'-CTG AGC GAC GAG TAC AAG AT-3'
	5'-GCG TCT CCA GGT TCG GCA-3'
	5'-FAM- CAA CAA CAT CGC G/TGT GCG CAA GAG CC-TAMRA-3'
DGAT1:	5'-CCG TGT TTG CTC TGG CAT C-3'
	5'-TGA CCT TCT TCC CTG TAG GAG-3'
	5'-FAM- TCC TAC CGG GAT GTC AAC CTG TGG-TAMRA-3'
DGAT2	5'-AGC TGT GCT CTA CTT CAC CT-3'
	5'-ACA CGG CCC AGT TT? GCA-3'
	5'-FAM-CCA CTG CGA TCT CCT GCC ACC TT--TAMRA-3'
HMGCoA synthase 2	5'-CTG GCC TCA CTT CTC TCT CA-3'
	3'-AAG CCT GAG CCG TAG GAG AA-3'
	5'FAM-CTC TGC CCA AGA ATT GGC TGG CTC C-TAMRA-3'
Hprt:	5'-GGA CTG ATT ATG GAC AGG AC-3'
	5'-CAG AGG GCC ACA ATG TGA T-3'
	5'-TET-CCT CCC ATC TCC TTC ATG ACA TCT CG-TAMRA-3'
leptin:	5'-AGC TGC AAG GCT CTG CTG-3'
	5'-CGA GTT GTT ATA GCC TCG GA-3'
	5'-FAM- TAG TCC CCA GAC GTG AGG AAC ACA G-TAMRA-3'
pancreatic lipase	5' GCC AGG ATG CCA GAA GAA TA-3'
	5'-ACA GGC AGC AAA GTT TCG AG-3'
	5'FAM-CTT CCC AGA TCC CGT CGA TGT CAA C-TAMRA-3'
PPARγ:	5'-AGA CAT GAG CCT TCA CCC C-3'
	5'-AGA AGG AAC ACG TTG TCA GC-3'
	5'-FAM- CAA GTC CTT GTA GAT CTC CTG GAG C-TAMRA-3'
SREBP1a:	5'-AGA TGT GCG AAC TGG ACA CA-3'
	5'-TGT CTC ACC CCC AGC ATA G-3'
	5'-FAM- AGA CAT GCT CCA GCT CAT CAA CAA CCA A-TAMRA-3'
SREBP1c:	F: ACG GAG CCA TGG ATT GCA C
	R: TGT CTC ACC CCC AGC ATA G
	5'-FAM- AGA CAT GCT CCA GCT CAT CAA CAA CCA A-TAMRA-3'
UCP1:	5'- TAT AGC CAC CAC AGA AAG CTT-3'
	5'-ACC AGC TCT GTA CAA TTG ATG-3'
	5'-FAM- CGA CCC CTA ATC TAA TGA GAA ATG TCA-TAMRA-3'
UCP2:	5'-ACA AGA CCA TTG CAC GAG AG-3'
	5'-ACC AGC TCA GCA CAG TTG A-3'
	5'-FAM- CTC CCA ATG TTG CCC GTA ATG CCA-TAMRA-3'
UCP3:	5'-ATC GCC AGG GAG GAA GGA G-3'
	5'-CAC CAT CTC AGC ACA GTT GA-3'
	5'-FAM- TGG CCC AAC ATC ACA AGA AAT GCC A-TAMRA-3'

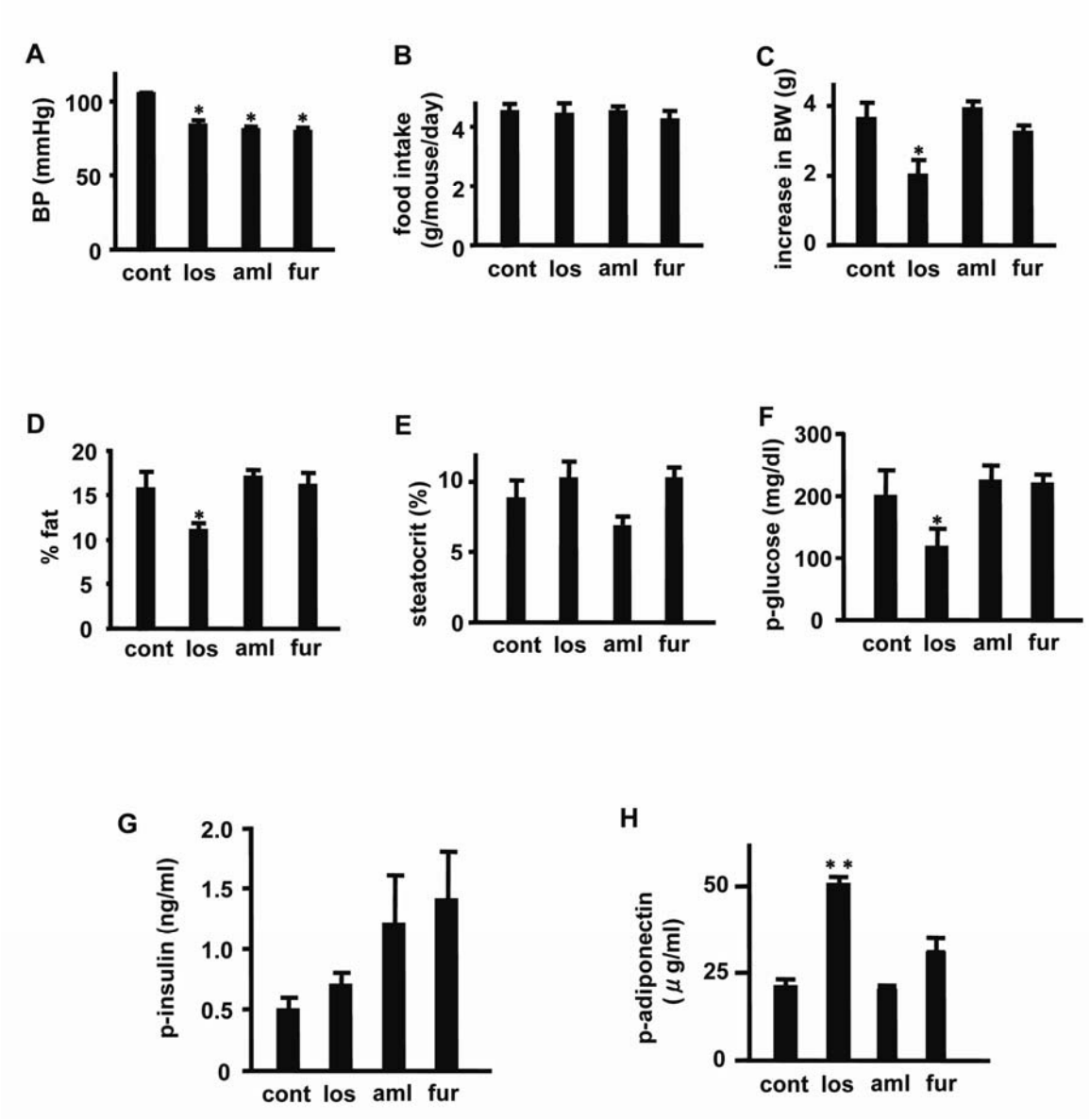

Figure S1. Losartan Replicates the Metabolic Phenotype of the *Ren1c-/-* Mice, but Amlodipine and Furosemide Do Not

Male WT mice 3 months of age were fed a high-fat diet with losartan (los) 0.45 g/l in drinking water, amlodipine (aml) 60 mg/kg food, furosemide (fur) 0.1 g/l in drinking water, or without drugs (cont). Blood pressure (A), food intake (B), increase in body weight (C), % body fat (D), fecal acid steatocrit (E), plasma glucose (F), plasma insulin (G), plasma adiponectin (H). Data were obtained 2 weeks after the treatment. $n \geq 5$ each. *$p < 0.05$, **$p < 0.0001$ compared to control. Error bars represent ± SEM in all panels.