

Targeting the Endocannabinoid/CB1 Receptor System for Treating Obesity in Prader-Willi Syndrome

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Supplementary Materials

Supplementary Fig. 1. Analysis of covariance (ANCOVA) for calculating metabolic data.

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Supplementary Table 1. Correlations among AEA, 2-AG, and AA and metabolic parameters in Israeli PWS patients.

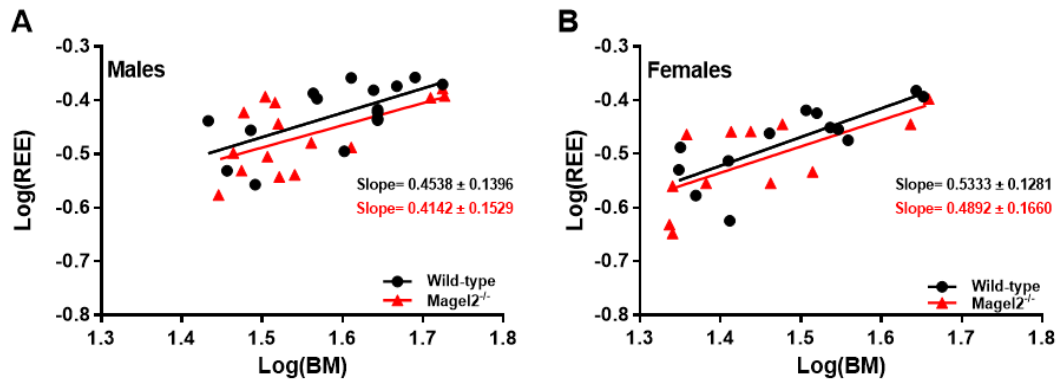
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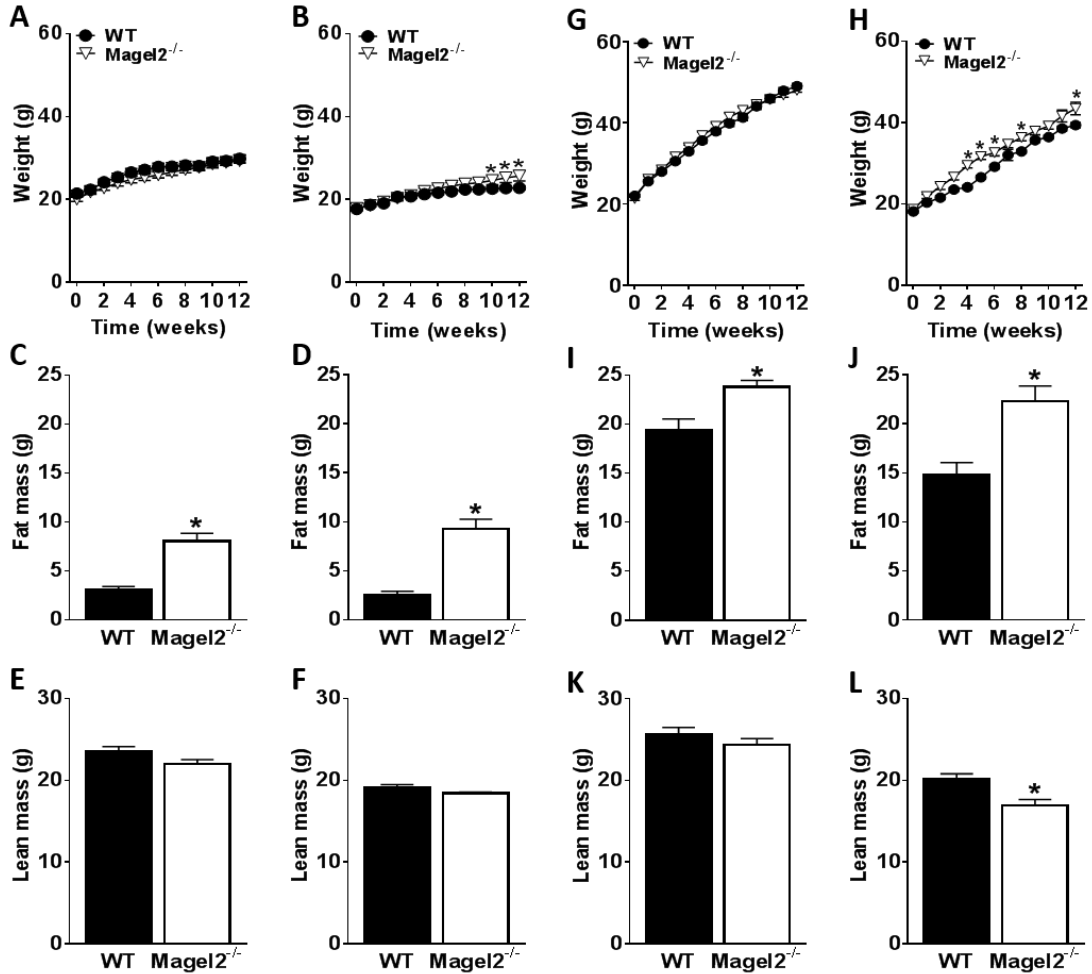
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Supplementary Fig. 1.



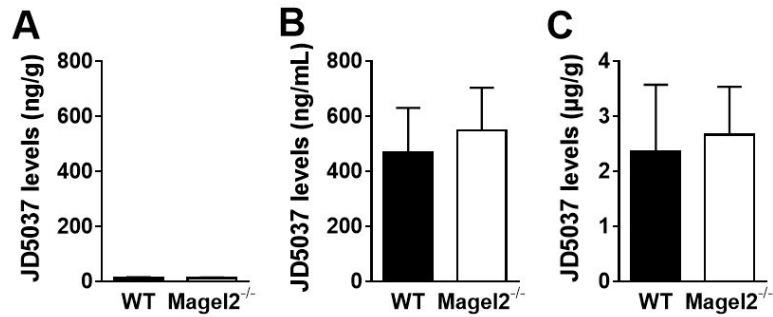
Analysis of covariance (ANCOVA) for calculating metabolic data. Logarithms of body mass and of the resting metabolic rate that were regressed against each other. The slopes of the regression curves were used as the power to which body mass was raised in order to yield the panels describing TEE, FO and CHO in *Figures 2 & 7*, in proportion to their metabolic rate.

Supplementary Fig. 2.



Altered body composition in *Magel2*-null mice fed either a STD or a HFD. *Magel2*-null mice and their littermate wild-type control animals were fed either a STD (A-F) or a HFD (G-L) for 12 weeks. Changes in body weight (A, B, G, H), fat mass (C, D, I, J), and lean mass (E, F, K, L) were measured in both male (A, C, E, G, I, K) and female (B, D, F, H, J, L) mice. Data represent the mean \pm SEM from 15-23 mice per group. * $P < 0.05$ relative to wild-type controls of the same sex.

Supplementary Fig. 3.



Low levels of JD5037 in the brain in *Magel2*-null and littermate control mice. Brain (A), serum (B), and liver (C) levels of JD5037 1 h following an acute administration at 3 mg/kg. Data represent the mean \pm SEM from 3 mice per group. * $P < 0.05$ relative wild-type controls.

Supplementary Table 1. Correlations among AEA, 2-AG, and AA and metabolic parameters in Israeli PWS patients

Parameter	AEA	2-AG	AA
Age	-0.41*	-0.26	-0.21
Weight	-0.25	-0.05	0.09
BMI	-0.18	-0.05	0.05
Insulin	-0.01	-0.04	0.37
Glucose	-0.03	0.33	0.07
Triglycerides	0.06	0.36	0.23
Cholesterol	0.17	0.04	0.38
HDL	-0.13	-0.51*	0.11
LDL	0.19	0.14	0.16
CRP	-0.12	0.10	0.00
HOMA	0.00	0.05	0.49*
Leptin	0.10	-0.26	0.31
Adiponectin	-0.29	-0.33	-0.36

*** $P < 0.05$**

Supplementary Table 2. eCB 'tone' in male *Magel2*-null mice and their littermate controls fed a STD

		Wild-type	<i>Magel2</i> -null	<i>P</i> Value
Blood	AEA	1.5 ± 0.1	1.3 ± 0.1	n.s.
	2-AG	74.2 ± 6.1	63.5 ± 3.9	n.s.
	AA	6.0 ± 0.9	5.0 ± 0.7	n.s.
Brain	AEA	51.2 ± 5.3	83.8 ± 12.7	0.04*
	2-AG	5.6 ± 0.6	4.3 ± 0.7	n.s.
	AA	6.2 ± 0.4	6.5 ± 0.5	n.s.
	<i>Cnr1</i>	1.0 ± 0.1	1.0 ± 0.1	n.s.
Epi. Fat	AEA	10.4 ± 0.5	11.8 ± 2.6	n.s.
	2-AG	1.4 ± 0.2	1.3 ± 0.1	n.s.
	AA	6.4 ± 0.8	3.3 ± 0.5	0.01*
	<i>Cnr1</i>	1.1 ± 0.2	0.8 ± 0.1	n.s.
RP. Fat	AEA	5.8 ± 0.6	8.9 ± 1.5	n.s.
	2-AG	2.3 ± 0.4	1.7 ± 0.1	n.s.
	AA	14.6 ± 1.9	10.4 ± 0.4	n.s.
	<i>Cnr1</i>	1.1 ± 0.2	0.7 ± 0.1	n.s.
SC. Fat	AEA	10.0 ± 0.6	9.2 ± 0.7	n.s.
	2-AG	3.0 ± 0.4	2.5 ± 0.7	n.s.
	AA	14.9 ± 1.9	9.2 ± 0.4	0.02*
	<i>Cnr1</i>	1.1 ± 0.2	0.7 ± 0.2	n.s.

Data represent the mean ± SEM from 4-5 animals per group. Values are expressed as pmol/mL or fmol/mg for AEA, pmol/mL or pmol/mg for 2-AG, and nmol/mL or pmol/mg for AA. *Cnr1* mRNA expression levels are expressed as relative values. Epididymal (Epi); Retroperitoneal (RP); Subcutaneous (SC); not significant (n.s.).

Supplementary Table 3. eCB 'tone' in female *Magel2*-null mice and their littermate controls fed a STD

		Wild-type	<i>Magel2</i>-null	<i>P</i> Value
Blood	AEA	7.4 ± 0.3	7.0 ± 0.4	n.s.
	2-AG	96.6 ± 11.8	85.4 ± 7.6	n.s.
	AA	11.6 ± 1.8	6.8 ± 1.0	n.s.
Brain	AEA	51.1 ± 8.0	60.8 ± 9.3	n.s.
	2-AG	6.9 ± 0.7	6.7 ± 0.6	n.s.
	AA	5.4 ± 0.4	3.7 ± 0.5	0.04*
	<i>Cnr1</i>	1.2 ± 0.1	1.0 ± 0.2	n.s.
Epi. Fat	AEA	7.8 ± 0.2	6.9 ± 0.6	n.s.
	2-AG	1.6 ± 0.2	1.3 ± 0.2	n.s.
	AA	8.8 ± 0.4	5.3 ± 0.9	0.01*
	<i>Cnr1</i>	1.0 ± 0.1	1.1 ± 0.2	n.s.
RP. Fat	AEA	16.2 ± 4.0	14.5 ± 4.2	n.s.
	2-AG	1.0 ± 0.1	1.0 ± 0.1	n.s.
	AA	8.2 ± 1.7	7.0 ± 1.7	n.s.
	<i>Cnr1</i>	1.1 ± 0.4	0.3 ± 0.1	n.s.
SC. Fat	AEA	10.0 ± 0.5	11.0 ± 1.8	n.s.
	2-AG	1.7 ± 0.2	1.5 ± 0.5	n.s.
	AA	15.2 ± 1.0	8.0 ± 1.5	0.01*
	<i>Cnr1</i>	1.0 ± 0.1	0.6 ± 0.2	n.s.

Data represent the mean ± SEM from 4-5 animals per group. Values are expressed as pmol/mL or fmol/mg for AEA, pmol/mL or pmol/mg for 2-AG, and nmol/mL or pmol/mg for AA. *Cnr1* mRNA expression levels are expressed as relative values. Epididymal (Epi); Retroperitoneal (RP); Subcutaneous (SC); not significant (n.s.).

Supplementary Table 4. eCB 'tone' in male *Magel2*-null mice and their littermate controls fed a HFD

		Wild-type	<i>Magel2</i>-null	<i>P</i> Value
Blood	AEA	3.2 ± 0.1	3.5 ± 0.3	n.s.
	2-AG	67.7 ± 4.4	66.1 ± 5.7	n.s.
	AA	10.6 ± 0.7	12.7 ± 1.8	n.s.
Brain	AEA	55.6 ± 2.8	44.7 ± 11.9	n.s.
	2-AG	5.2 ± 0.4	5.3 ± 0.6	n.s.
	AA	6.2 ± 0.6	6.5 ± 0.4	n.s.
	<i>Cnr1</i>	1.0 ± 0.1	0.8 ± 0.1	n.s.
Epi. Fat	AEA	18.3 ± 1.7	14.2 ± 2	n.s.
	2-AG	3.5 ± 0.9	1.818 ± 0.7	n.s.
	AA	4.3 ± 0.3	4.5 ± 0.8	n.s.
	<i>Cnr1</i>	1.2 ± 0.3	1.0 ± 0.08	n.s.
RP. Fat	AEA	14.3 ± 1.7	11.1 ± 2.7	n.s.
	2-AG	1.9 ± 0.2	1.5 ± 0.4	n.s.
	AA	10.2 ± 1.1	8.8 ± 1.5	n.s.
	<i>Cnr1</i>	1.0 ± 0.1	1.0 ± 0.2	n.s.
SC. Fat	AEA	17.0 ± 2.2	13.4 ± 2.9	n.s.
	2-AG	1.5 ± 0.2	1.0 ± 0.2	n.s.
	AA	4.3 ± 0.7	7.8 ± 0.9	0.01*
	<i>Cnr1</i>	1.1 ± 0.2	0.6 ± 0.2	n.s.

Data represent the mean ± SEM from 4-5 animals per group. Values are expressed as pmol/mL or fmol/mg for AEA, pmol/mL or pmol/mg for 2-AG, and nmol/mL or pmol/mg for AA. *Cnr1* mRNA expression levels are expressed as relative values. Epididymal (Epi); Retroperitoneal (RP); Subcutaneous (SC); not significant (n.s.).

Supplementary Table 5. eCB 'tone' in female *Magel2*-null mice and their littermate controls fed a HFD.

		Wild-type	<i>Magel2</i>-null	<i>P</i> Value
Blood	AEA	8.6 ± 0.2	9.3 ± 0.6	n.s.
	2-AG	107.3 ± 8.6	108.7 ± 7.7	n.s.
	AA	16.2 ± 2.3	12.7 ± 2.7	n.s.
Brain	AEA	31.2 ± 3.4	44.4 ± 2.6	0.01*
	2-AG	6.9 ± 0.4	5.8 ± 0.3	n.s.
	AA	3.6 ± 0.5	4.3 ± 0.5	n.s.
	<i>Cnr1</i>	1.0 ± 0.1	1.2 ± 0.2	n.s.
Epi. Fat	AEA	14.7 ± 1.8	13.8 ± 0.2	n.s.
	2-AG	0.9 ± 0.1	0.9 ± 0.06	n.s.
	AA	6.1 ± 0.7	5.7 ± 0.7	n.s.
	<i>Cnr1</i>	1.1 ± 0.2	0.9 ± 0.1	n.s.
RP. Fat	AEA	15.0 ± 1.1	18.1 ± 1.4	n.s.
	2-AG	0.6 ± 0.1	0.8 ± 0.1	n.s.
	AA	7.9 ± 1.5	6.9 ± 0.7	n.s.
	<i>Cnr1</i>	1.2 ± 0.3	1.1 ± 0.4	n.s.
SC. Fat	AEA	19.8 ± 4.4	12.1 ± 2.5	n.s.
	2-AG	0.9 ± 0.1	0.9 ± 0.1	n.s.
	AA	9.6 ± 1.5	9.0 ± 1.1	n.s.
	<i>Cnr1</i>	1.1 ± 0.3	0.5 ± 0.2	n.s.

Data represent the mean ± SEM from 4-5 animals per group. Values are expressed as pmol/mL or fmol/mg for AEA, pmol/mL or pmol/mg for 2-AG, and nmol/mL or pmol/mg for AA. *Cnr1* mRNA expression levels are expressed as relative values. Epididymal (Epi); Retroperitoneal (RP); Subcutaneous (SC); not significant (n.s.).