A Dual Inhibition of Cannabinoid-1 Receptor and iNOS Attenuates Obesity-induced Chronic Kidney Disease

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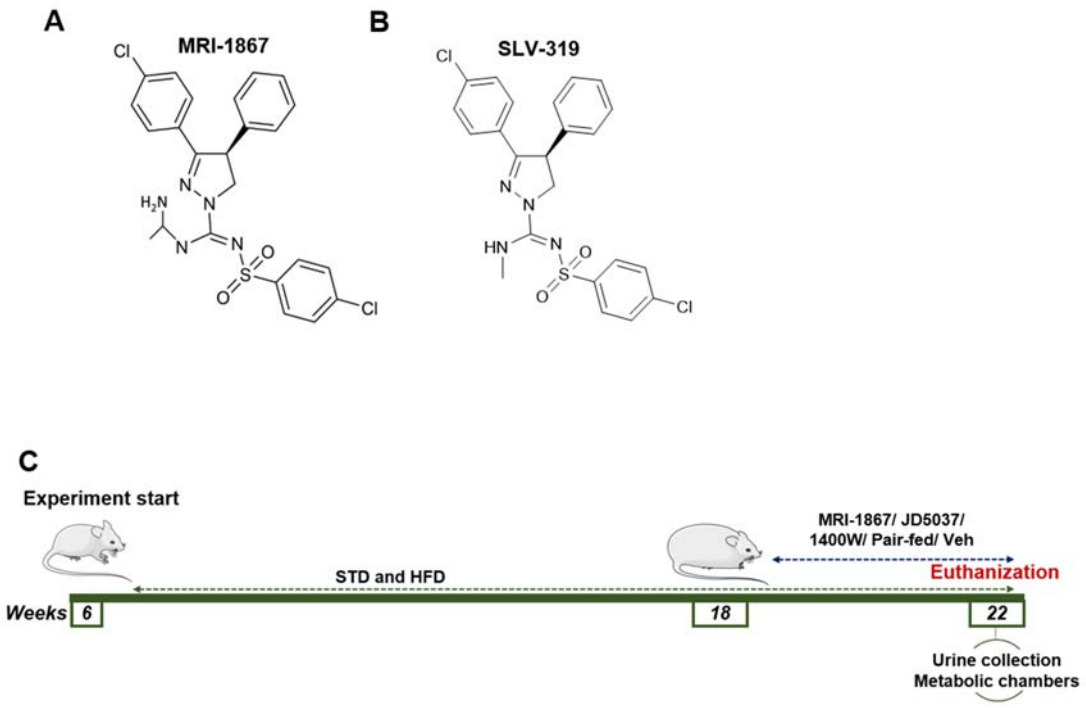
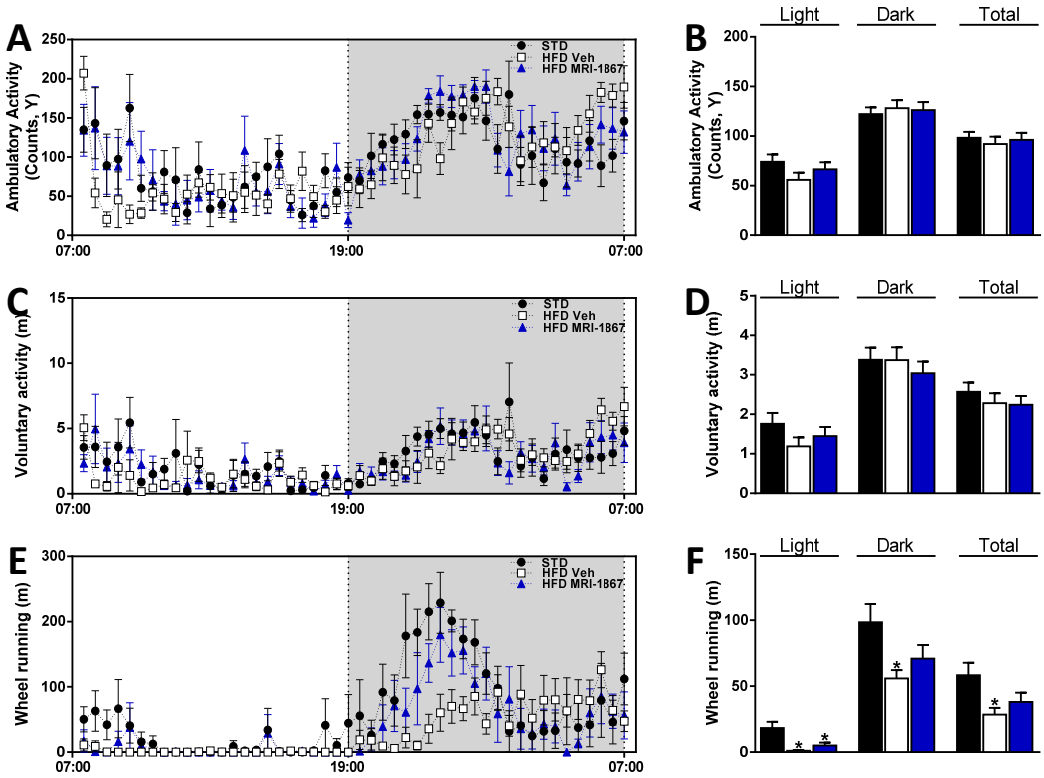
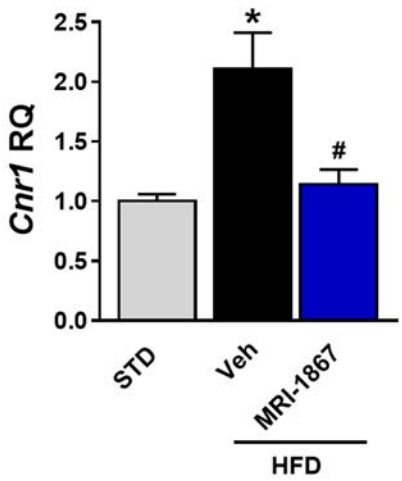
Supplementary Information



Supplementary Figure 1. MRI-1867 chemical structure and experimental protocol. The chemical structure of MRI-1867, as described previously (Cinar et al., JCI Insight 2016) (**A**), derived from the globally acting CB_1R antagonist, SLV-319 (**B**). Experimental protocol (**C**): Six-week old male C57Bl/6J mice were fed either a high-fat diet (HFD) or a standard diet (STD) for 18 weeks, and then treated chronically for 28 days by oral gavage with either vehicle (Veh; 1% Tween80, 4% DMSO, 95% Saline; n=8 for STD and n=14 for HFD), peripherally restricted dual CB_1R/iNOS antagonist, MRI-1867 (3 mg kg^{-1}; n=14), peripherally restricted CB_1R antagonist, JD5037 (3 mg kg^{-1}; n=8), or iNOS inhibitor, 1400W (10 mg kg^{-1}; n=7). A parallel HFD group treated with Veh was pair-fed to the MRI-1867 group (n=7).



Supplementary Figure 2. MRI-1867 does not affect ambulatory and voluntary activities in diet-induced obese mice. Mice on STD or HFD for 18 weeks were treated with vehicle (Veh) or MRI-1867 (3 mg kg^{-1}) orally for 28 days. No significant changes in ambulatory (**A**, **B**) and voluntary (**C, D**) activities, as well as wheel running capabilities (**E, F**) were observed for DIO mice treated with Veh or MRI-1867. Data represent the mean ± SEM from 8-10 mice per group, and were analyzed by ANOVA followed by Bonferroni post hoc test. *P<0.05 relative to animals on STD.



Supplementary Figure 3. MRI-1867 reduces CB_1R in DIO mice. The elevated CB_1R mRNA expression levels in DIO mice were normalized by chronic MRI-1867 treatment (3 mg kg^{-1}). Data represent the mean ± SEM from 8-14 mice per group, and were analyzed by ANOVA followed by Bonferroni post hoc test. *P<0.05 relative to animals on STD; #P<0.05 relative to animals on the same diet.

Supplementary Table 1. Primer information.

Gene	Forward	Reverse
mus musculus Kim1	CCACCAGCTCACCATTGTACT	TCTGCTTGGACTTCCTTTTCA
mus musculus β-Actin	GGC TGT ATT CCC CTC CAT CG	CCA GTT GGT AAC AAT GCC ATG
mus musculus Mcp1	GCATTAGCTTCAGATTTA	TTAAAAACCTGGATCGGAACCAA
mus musculus Adipoq	TCCGGGACTCTACTACTTCTC	AACGGCCTTGTCCTTCTTGA
mus musculus Cnr1	AAGTCGATCTTAGACGGCCTT	TCCTAATTTGGATGCCATGTCTC
homo sapience ADIPOQ	CCCAAAGAGGAGAGAGGAAGCT	GCCAGAGCAATGAGATGCAA
homo sapience ADIPOR1	AATTCCTGAGCGCTTCTTTCCT	CATAGAAGTGGACAAAGGCTGC
homo sapience ADIPOR2	TGCAGCCATTATAGTCTCCCAG	GAATGATTCCACTCAGGCCTAG
homo sapience PDGFa	GGCTGGCTCGAAGTCAGATC	CCTCAGCCCCTACGGAGTCT
homo sapience FGF2	TCCAGTTGGTATGTGGCACTGA	CAGTATGGCCTTCTGTCCAGGTC
homo sapience MitoDNA	CACTTTCCACACAGACATCA	TGGTTAGGCTGGTGTTAGGG
homo sapience β2M	TGTTCCTGCTGGGTAGCTCT	CCTCCATGATGCTGCTTACA
homo sapience RPLP0	CCAACTACTTCCTTAAGATCATCCAA	ACATGCGGATCTGCTGCTGCA

Supplementary Table 2. Primer information.

Gene	QuantiTect Primer Assays (Qiagen, Germany)
mus musculus Col1a1	QT00162204
mus musculus Timp1	QT00996282
mus musculus Nos2	QT00100275
mus musculus Adipor1	QT00154217
mus musculus Adipor2	QT00165326