Supplementary Methods

Hepatic de novo fatty acid synthesis. Rate of de novo fatty acid synthesis was measured in mouse isolated hepatocytes by the incorporation of ^3H_2O into hepatic fatty acids as described (1) with minor modifications. Briefly, hepatocytes were isolated, plated in 6-well collagen-coated plates and cultured for 24 h before the addition of vehicle, the indicated drugs or their combinations, with the CB_1R antagonists added 3 h prior to the addition of 2-AG or vehicle. Twenty four hours later, 167 μCi ^3H_2O was added to each well for 60 min and the reaction was then terminated by the addition of chloroform/methanol (1:1). Fatty acids were extracted with ether and newly synthesized species were quantified by liquid scintillation spectrometry. The rate of lipogenesis was expressed as nmol ^3H_2O incorporated into fatty acids per min per 10^8 cells.

Interaction of AM6545 with P-glycoprotein (P-gp, ABCB1). Human epidermoid carcinoma parental KB-3-1– and P-gp (ABCB1)-overexpressing KB-V1 cells were maintained in DMEM supplemented with 10% fetal bovine serum, penicillin and streptomycin, and KB-V1 cells were grown in media containing 1 μg/ml vinblastine, as described (2). Inhibition of P-gp-mediated transport was determined by flow cytometry using fluorescent calcein accumulation as described (3). Briefly, 300,000 cells were incubated at 37°C for 10 min in the presence of 0.5 μM calcein-AM in the presence or absence of the indicated concentration of AM6545, or the P-gp-specific inhibitor Tariquidar (XR 9576; 5 μM) as positive control. The interaction of AM6545 at drug-substrate binding site was determined by inhibition of binding of photoaffinity labeled substrate [125I]-iodoarylazidoprazosin to P-gp in crude membranes as described (4).

Upper gastrointestinal transit. Upper gastrointestinal transit was assessed as described previously (5) with modifications. Briefly, 12 week old male mice were fasted overnight (water *ad libitum*), weighted and administered intraperitonealy with vehicle, AEA (10 mg/kg), rimonabant (10

mg/kg), AM6545 (10 mg/kg) or combination as described in the figure legend 1 h before the oral administration of a black marker (10% charcoal suspension in 5% gum arabic, 0.1 mL per 10 g BW). Thirty min later, the mice were killed and the gastrointestinal tract was removed immediately. The distance travelled by the marker was measured and expressed as a percentage of the total length of the small intestine from pylorus to caecum.

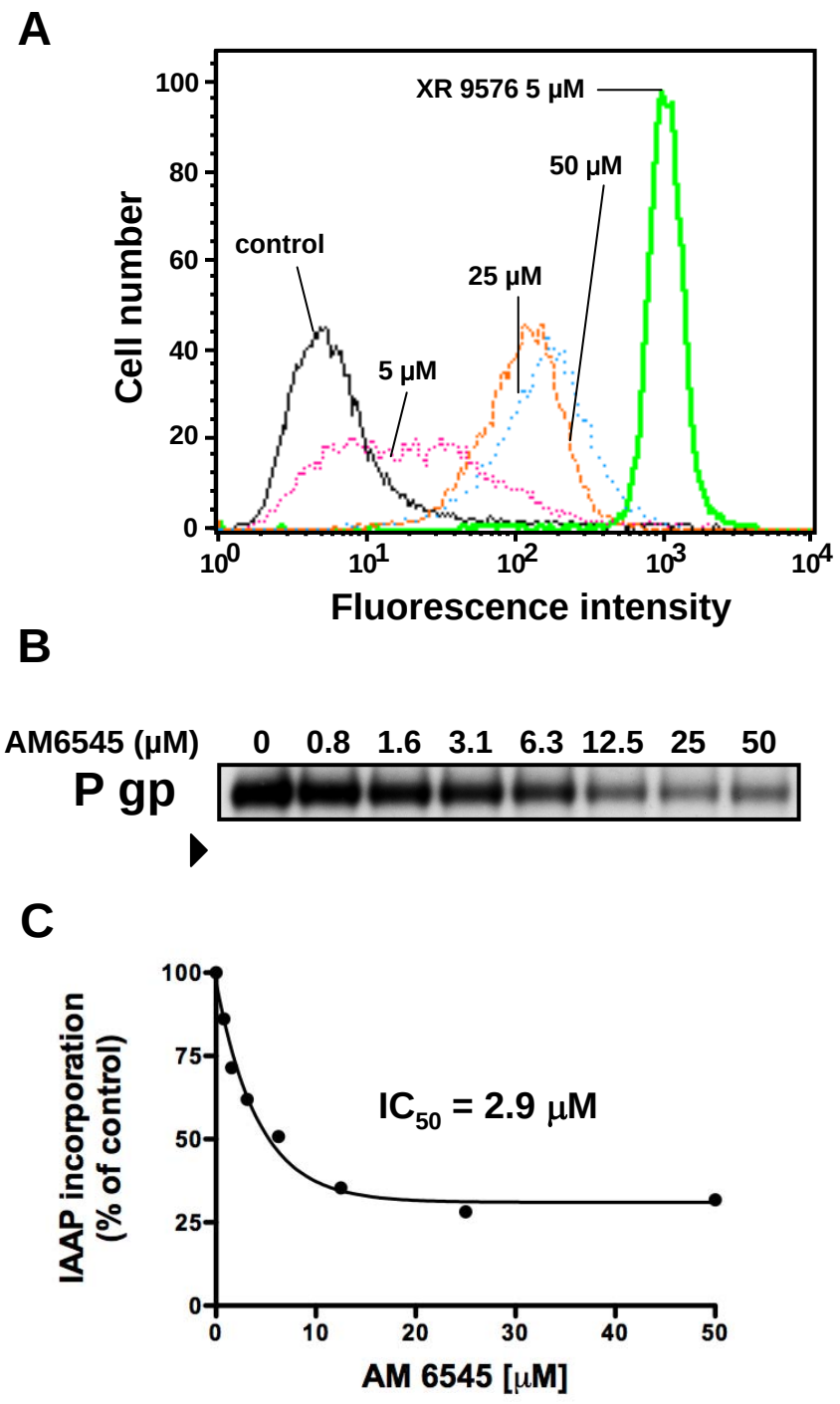
Intestinal transport. Intestinal permeability for AM6545 was assessed in monolayer cultures of Caco-2 cells, as described (6). Briefly, AM6545 at 50 μM was incubated for 2 hours on either side of the monolayer, and its concentration on both sides was determined using LC-MS/MS and used to calculate the efflux ratio.

Insulin resistance and insulin sensitivity index. The homeostasis model assessment was used to calculate the insulin resistance (HOMA-IR) index using the formula: HOMA-IR= fasting glucose (mmol/L) x plasma insulin (mU/L) /22.5. Fasting glucose and insulin levels were used to calculate relative insulin sensitivity index (ISI) as 1/(glucose x insulin) x 1000, with glucose expressed as mg/dL and insulin as mU/L (7).

1. **Lowenstein JM, Brunengraber H, Wadke M. Measurement of rates of lipogenesis with deuterated and tritiated water. *Methods Enzymol.*1975; 35279-287.**
2. **Shen DW, et al. Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification. *Science.*1986; 232(4750):643-645.**
3. **Shukla S, Robey RW, Bates SE, Ambudkar SV. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. *Drug Metab Dispos.*2009; 37(2):359-365.**
4. **Sauna ZE, Ambudkar SV. Evidence for a requirement for ATP hydrolysis at two distinct steps during a single turnover of the catalytic cycle of human P-glycoprotein. *Proc Natl Acad Sci U S A.*2000; 97(6):2515-2520.**
5. **Izzo AA, Mascolo N, Pinto L, Capasso R, Capasso F. The role of cannabinoid receptors in intestinal motility, defaecation and diarrhoea in rats. *Eur J Pharmacol.*1999; 384(1):37-42.**

6. **van Breemen RB, Li Y. Caco-2 cell permeability assays to measure drug absorption. *Expert Opin Drug Metab Toxicol.*2005; 1(2):175-185.**
7. **Zhang N, Huan Y, Huang H, Song GM, Sun SJ, Shen ZF. Atorvastatin improves insulin sensitivity in mice with obesity induced by monosodium glutamate. *Acta Pharmacol Sin.*2010; 31(1):35-42.**

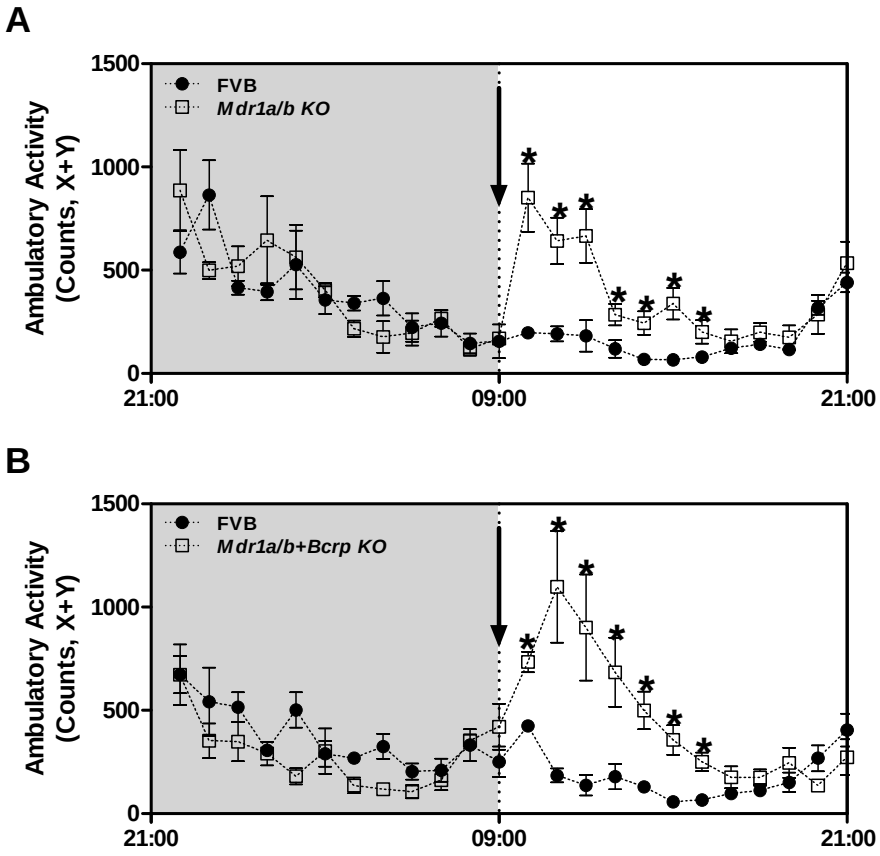
Supplementary figure 1



Interaction of AM6545 with P-glycoprotein

A: Effect of AM6545 on the accumulation of calcein (0.2 µM) P-gp expressing KB-V1 cells. **B**: Effect of AM6545 on photoaffinity labeling of P-gp with $^{[125I]}$IAAP. **C**: The incorporation of $^{[125I]}$IAAP into P-gp in the presence of AM6545, as quantified from the autoradiogram in **B**.

Supplementary figure 2

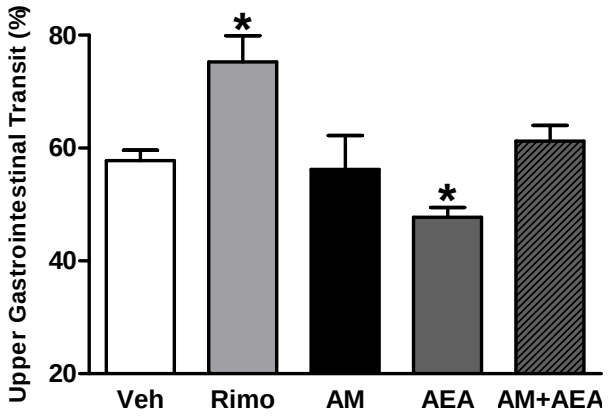


AM6545 increases ambulatory activity in P-glycoprotein deficient mice.

AM6545 causes a CB_1R-mediated acute increase in ambulatory activity in P-gp (mdr1a/b) knockout mice (**A**) and in mice deficient in both P-gp and Bcrp/Abcg2 (**B**). Disruption of infrared xy beams following ip. injection of 10 mg/kg AM6545 in mice.

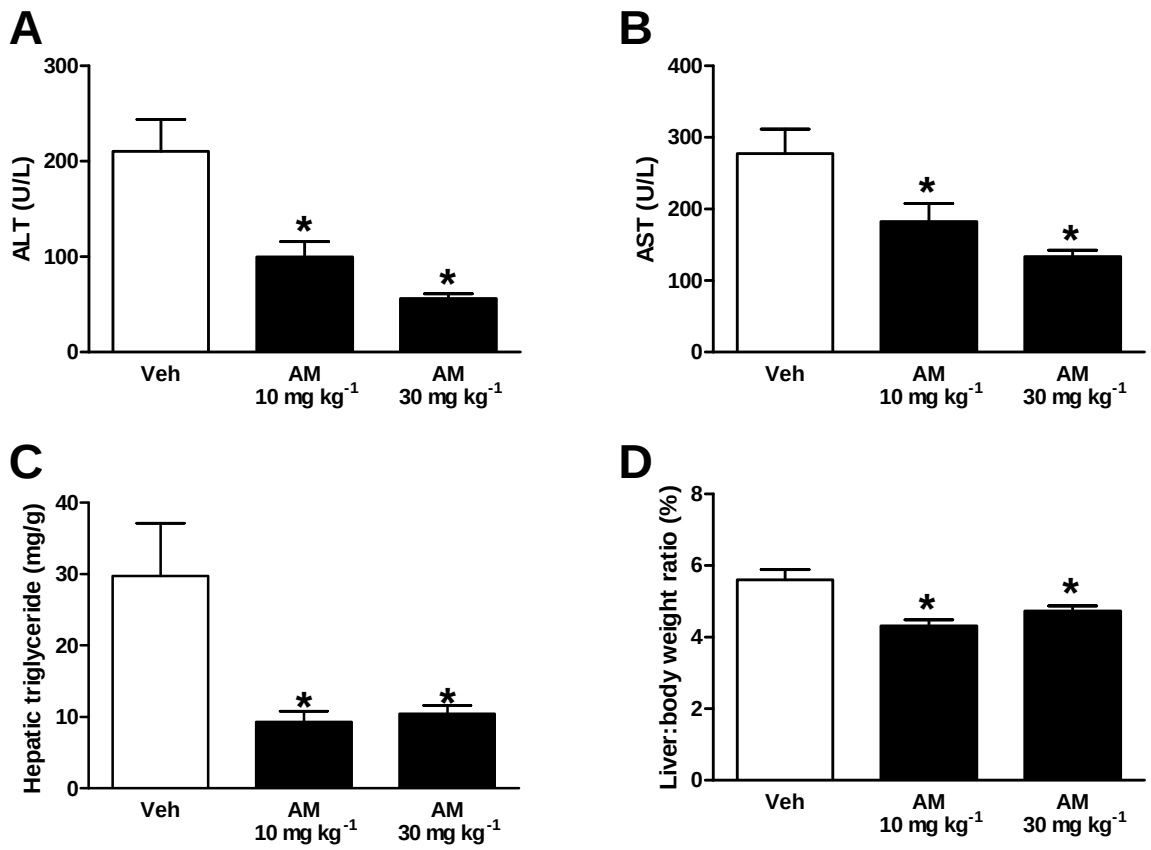
Results are mean ± SEM of 4 mice in each group. *P<0.01 relative to corresponding time point following AM6545 administration to the wild-type control FVB mice. Note that AM6545 was inactive in wild-type FVB mice, similar to its lack of effect in wild-type C57Bl6 mice (text Fig. 2D).

Supplementary figure 3



Effect of CB_1R agonist or antagonists on propulsive activity in mouse small intestine.
Drugs or vehicle were administered ip. 1 h prior to oral administration of the marker (10% charcoal suspension in 5% gum arabic). Thirty min later, mice were killed and distance travelled by the head of the marker, between the pylorus and the caecum, was measured and expressed as percent of total length of the small intestine. Note that rimonabant (Rimo, 10 mg/kg) but not AM6545 (AM, 10 mg/kg) increased GI transit, and that AM6545 completely inhibited the decrease in GI transit caused by a maximally effective dose of anandamide (AEA, 10 mg/kg).
Results are mean ± SEM of 5-9 mice in each group. *$P<0.05$ vs. vehicle.

Supplementary figure 4

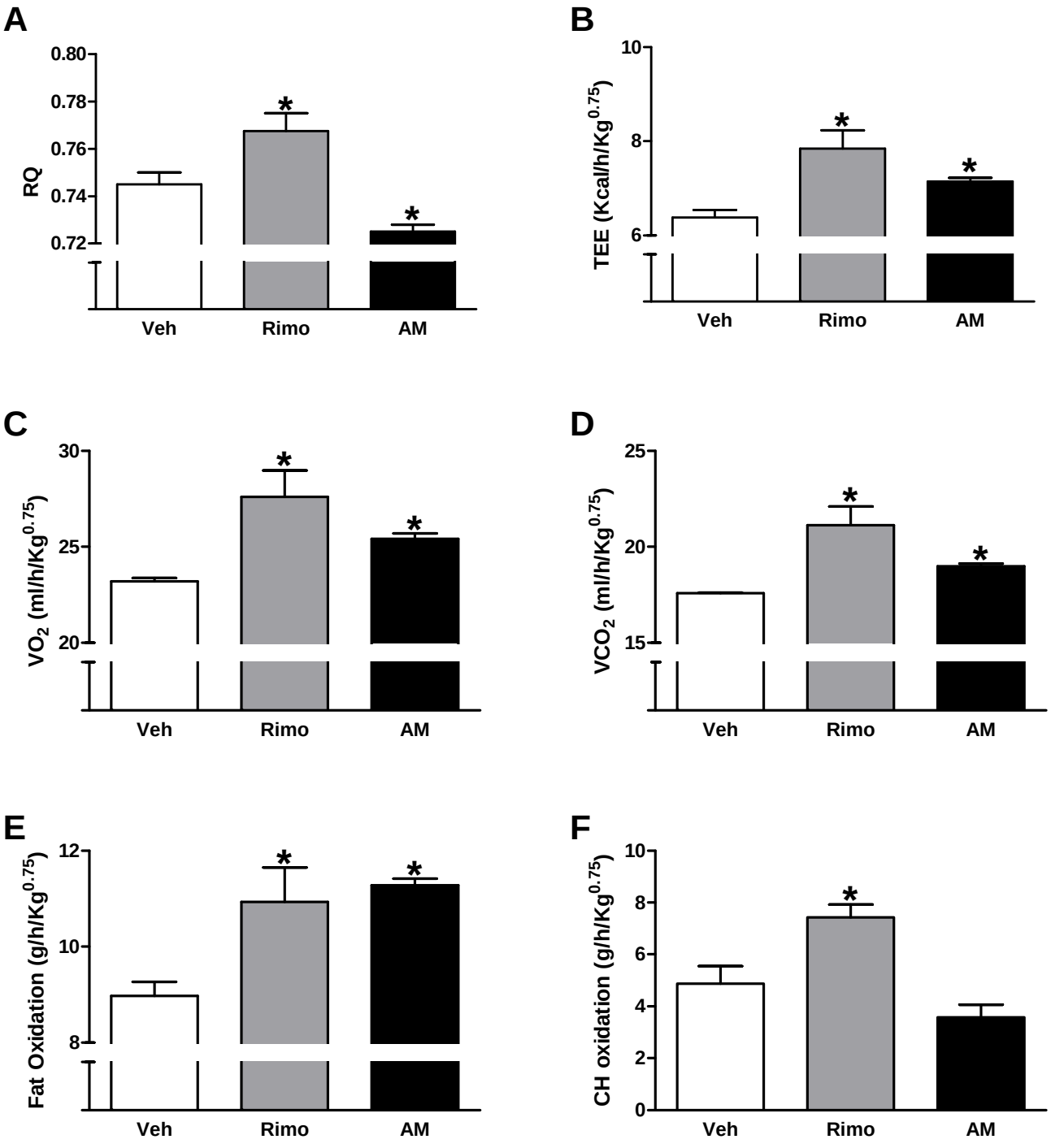


Oral treatment with AM6545 prevents diet-induced hepatic steatosis.

Oral administration of AM6545 to DIO mice at a dose of 10 or 30 mg/kg for 14 days prevents diet-induced hepatic steatosis and hepatocellular damage, as reflected by the reduction in serum ALT (**A**), AST (**B**), hepatic triglyceride content (**C**) and liver-to-body weight ratio (**D**).

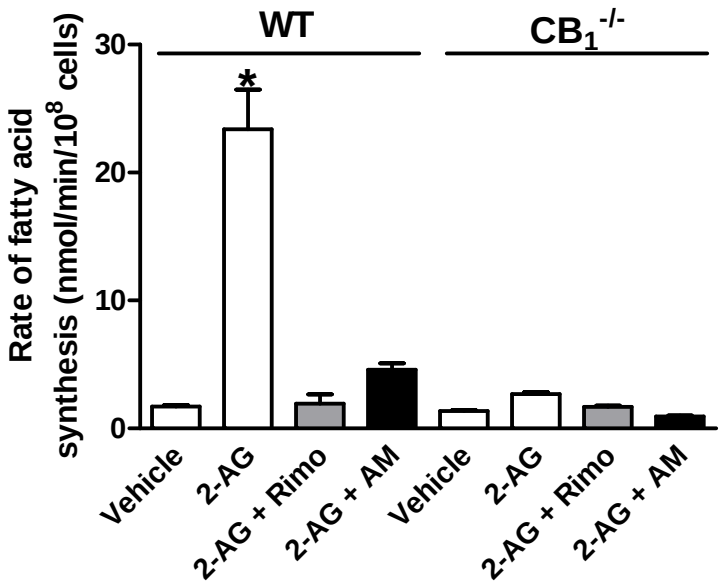
Data are mean ± SEM from 5-6 mice in each group. *P < 0.05 relative to vehicle group on HFD.

Supplementary figure 5



Effect of chronic treatment with AM6545 or rimonabant on metabolic parameters.
Daily treatment with AM6545 or rimonabant (10 mg/kg ip.) for 28 days induces changes in RQ (**A**), total energy expenditure (**B**), VO_2 (**C**), VCO_2 (**D**), fat (**E**) and carbohydrate (**F**) oxidation, as measured by indirect calorimerty over 24 h period at the end of the treatment. Data are mean ± SEM from 4-5 mice per condition. *P < 0.05 relative to vehicle group on HFD.

Supplementary figure 6

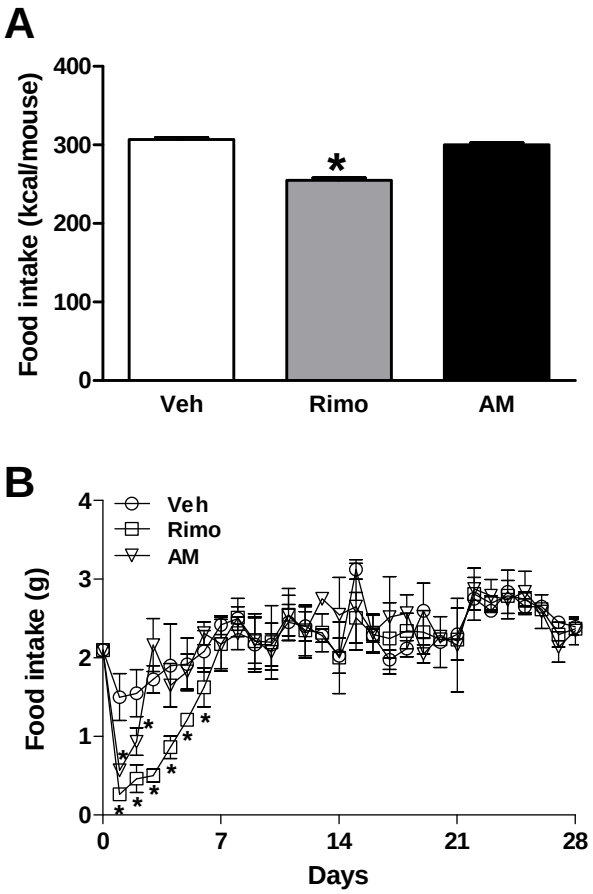


Effect of AM6545 on the rate of *de novo* hepatic lipogenesis in mice.

Hepatocytes were isolated from wild-type (WT) and $CB_1^{-/-}$ mice, and grown and treated as described in the methods. *De novo* lipogenesis was measured by the rate of ^3H_2O incorporation into hepatic fatty acids. Note that 2-AG (10 µM) increases lipogenesis, whereas rimonabant or AM6545 (1µM) inhibits this effect in WT but not in $CB_1^{-/-}$ mice.

Data are mean ± SEM from 6 replicates per condition. *P< 0.05 relative to vehicle group in each strain (ANOVA followed by Bonferroni's post-hoc test).

Supplementary figure 7

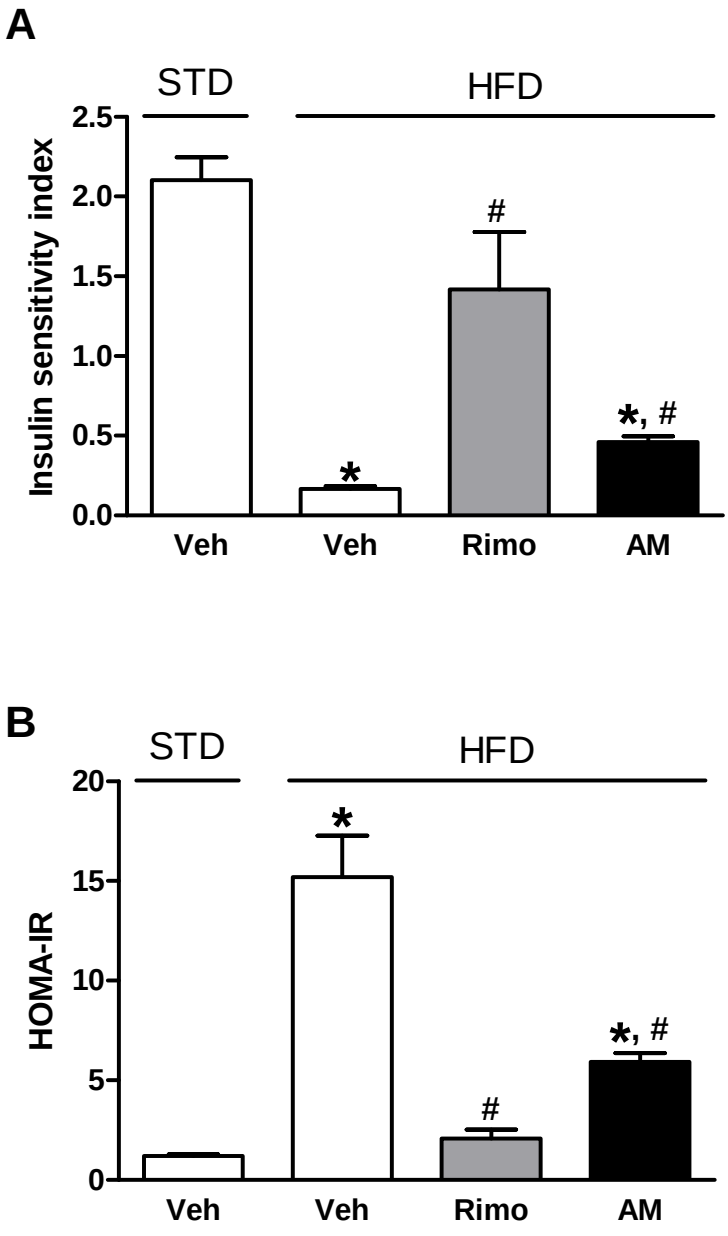


Effects of chronic treatment of DIO mice with AM6545 or rimonabant on total caloric intake (**a**) and daily food intake (**b**).

Mice were fed a high fat diet for 14 weeks before treatment with vehicle or 10 mg/kg/day ip. of either drug for 28 days.

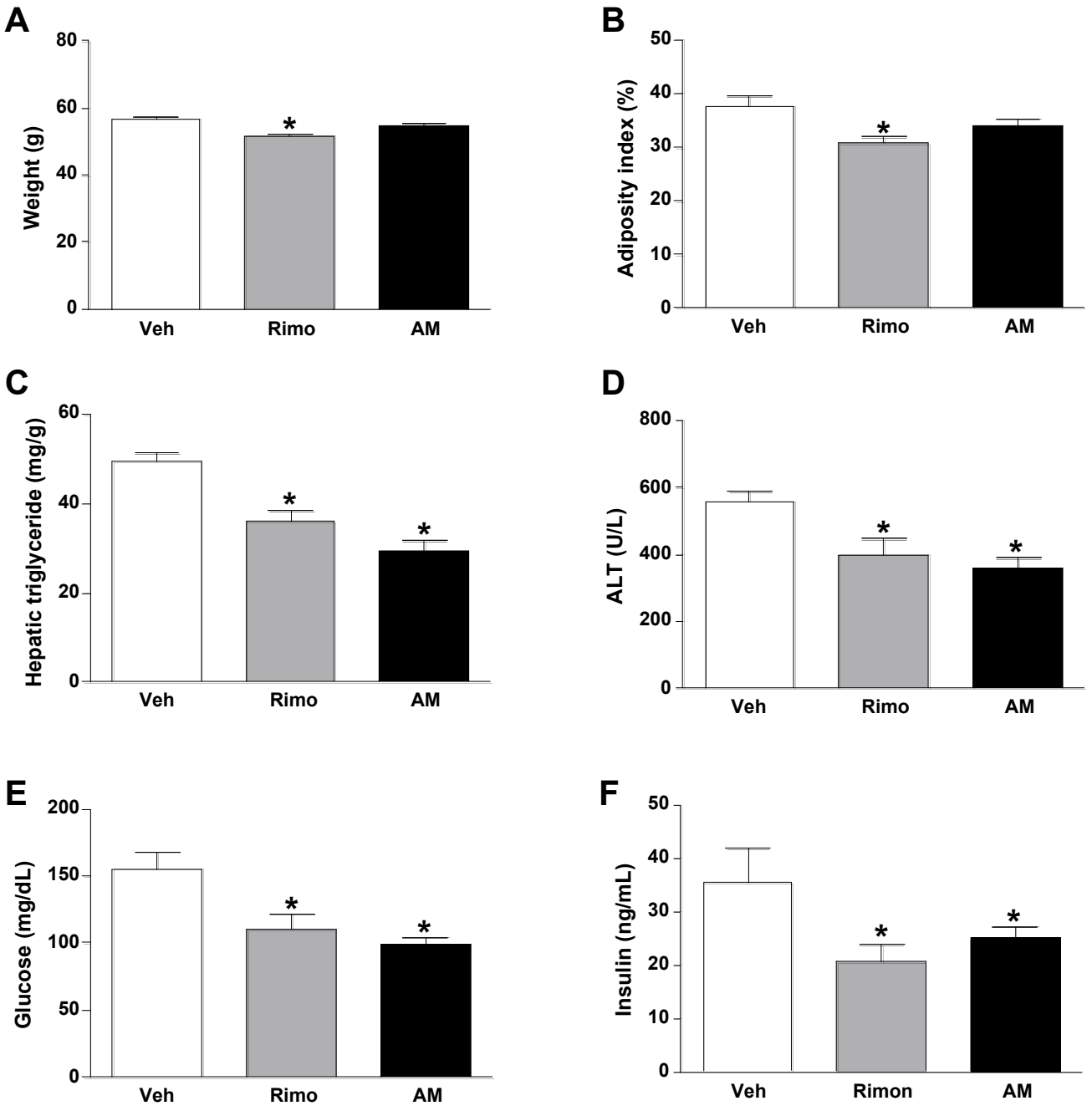
Data are mean ± SEM from 6-12 mice per condition. *P< 0.05 vs. vehicle.

Supplementary figure 8



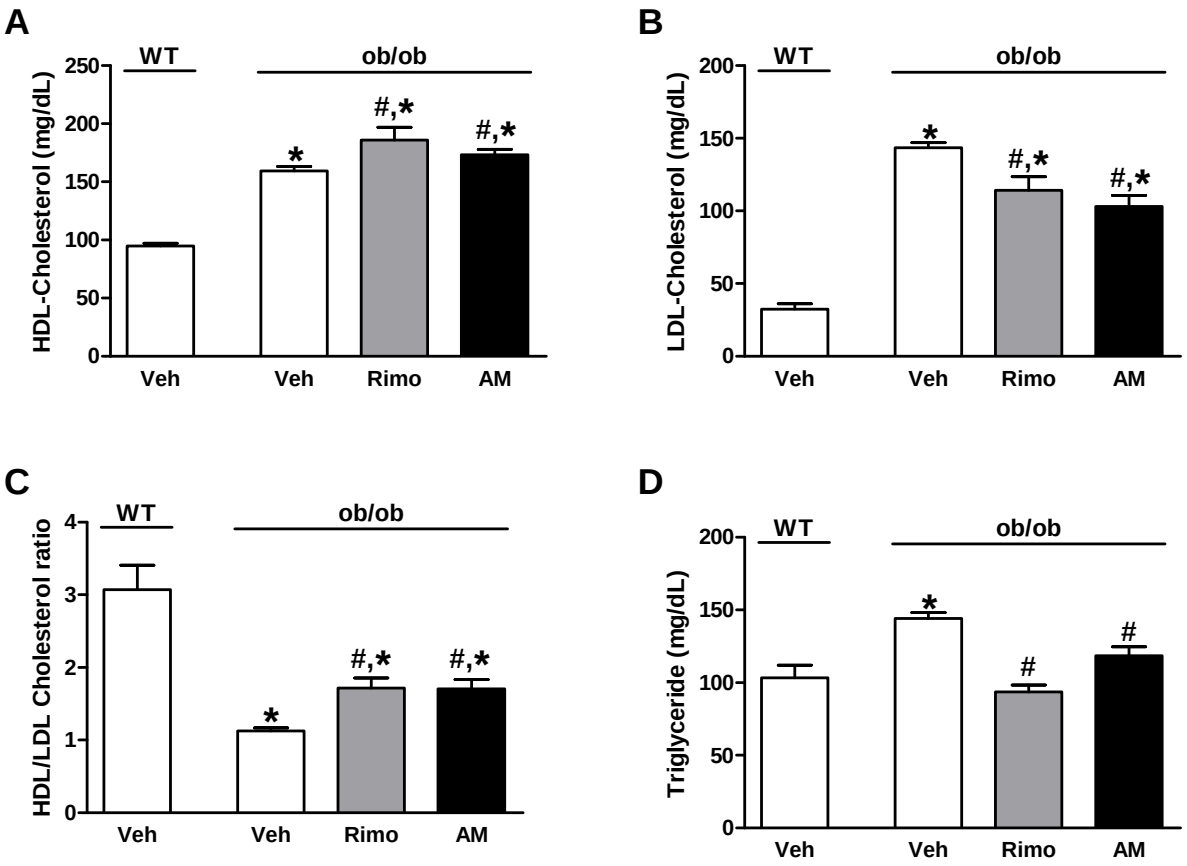
Effects of rimonabant and AM6545 on insulin resistance indices in DIO mice.
Mice were on STD or HFD for 14 weeks before treatment with 10 mg/kg/day ip. of either rimonabant or AM6545 for an additional 28 days. Both insulin sensitivity index and HOMA-IR were calculated from fasting glucose and insulin levels as described in the methods. Data are mean ± SEM from 8-12 mice per condition. *P<0.05 relative to vehicle group on STD; #P<0.05 relative to vehicle group on HFD

Supplementary figure 9



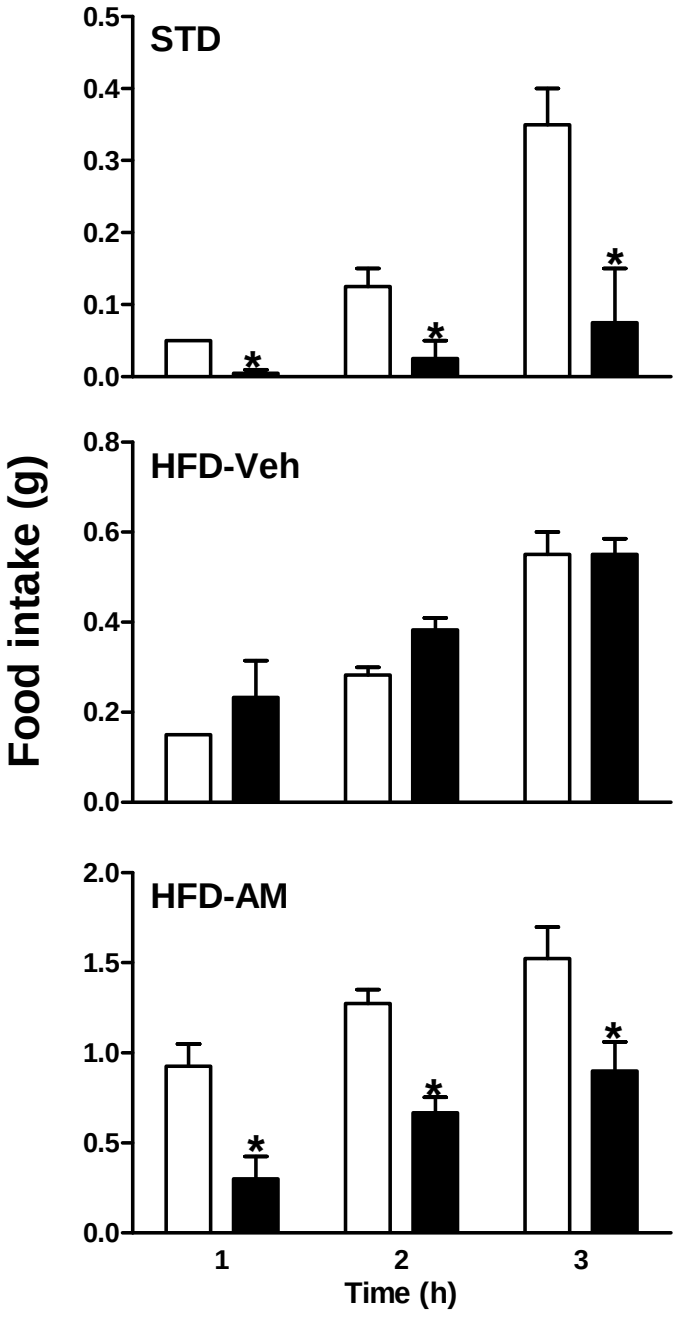
Metabolic effects of chronic treatment with AM6545 or rimonabant in *ob/ob* mice.
Adult male *ob/ob* mice were treated with AM6545 or rimonabant (10 mg/kg i.p.) for 28 days. Note that only rimonabant reduced body weight (**A**) and adiposity index (**B**). Both drugs were effective in reducing hepatic triglyceride (**C**), serum ALT (**D**), glucose (**E**) and insulin (**F**) levels. Data are mean ± SEM from 6 mice per condition. *P < 0.05 relative to vehicle group on HFD.

Supplementary figure 10



Rimonabant and AM6545 improve plasma lipid profile in ob/ob mice.
Both rimonabant and AM6545 (10 mg/kg/day ip for 28 days) increase HDL cholesterol (**A**) and decrease LDL cholesterol (**B**) levels resulting in increased HDL:LDL cholesterol ratio (**C**) and normalize serum triglyceride levels (**D**) in *ob/ob* mice. Data are mean ± SEM from 6 mice per condition. * $P < 0.05$ relative to value in lean controls; # $P < 0.05$ relative to corresponding value in vehicle-treated *ob/ob* mice.

Supplementary figure 11



AM6545 improve leptin-induced inhibition of food intake in DIO mice.

Short-term treatment with AM6545 (10 mg/kg ip. for 9 days) increase leptin-induced inhibition of acute food intake. Mice were kept on HFD or STD for 16 weeks, then treated with AM6545 or vehicle for 9 days. After the last injection, mice were fasted for 24 h then injected with leptin (10 mg/kg ip.) and acute food intake was measured. Data are mean ± SEM from 4-6 mice per condition. * $P < 0.05$ relative to value in vehicle treated group.