Role of adiponectin in the metabolic effects of cannabinoid type 1 receptor blockade in mice with diet-induced obesity

Joseph Tam, Grzegorz Godlewski, Brian J. Earley, Liang Zhou, Tony Jourdan, Gergö Szanda, Resat Cinar, and George Kunos

Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland

Submitted 5 September 2013; accepted in final form 25 December 2013

Tam J, Godlewski G, Earley BJ, Zhou L, Jourdan T, Szanda G, Cinar R, Kunos G. Role of adiponectin in the metabolic effects of cannabinoid type 1 receptor blockade in mice with diet-induced obesity. Am J Physiol Endocrinol Metab 306: E457–E468, 2014. First published December 31, 2013; doi:10.1152/ajpendo.00489.2013.—The adipocyte-derived hormone adiponectin promotes fatty acid oxidation and improves insulin sensitivity and thus plays a key role in the regulation of lipid and glucose metabolism and energy homeostasis. Chronic cannabinoid type 1 (CB1) receptor blockade also increases lipid oxidation and improves insulin sensitivity in obese individuals or animals, resulting in reduced cardiometabolic risk. Chronic CB1 blockade reverses the obesity-related decline in serum adiponectin levels, which has been proposed to account for the metabolic effects of CB1 antagonists. Here, we investigated the metabolic actions of the CB1 inverse agonist rimonabant in high-fat diet (HFD)-induced obese mice and wild-type littermate controls. Rimonabant treatment improved obesity and hormonal/metabolic consequences were indistinguishable in the two strains. Daily treatment of obese mice with rimonabant for 7 days resulted in significant and comparable reductions in body weight, increased insulin and leptin sensitivity, and improved glycemic control, is mediated by adiponectin.

ADIPOSE TISSUE IS NO LONGER CONSIDERED a passive energy store but rather a complex and highly active metabolic and endocrine organ that produces and secretes biologically active substances known as “adipokines” (41). Among them, adiponectin, a 30-kDa polypeptide that circulates at high levels in the blood, has been implicated in the maintenance of energy homeostasis and the prevention of several metabolic diseases. Indeed, serum levels of adiponectin are reduced in individuals with obesity and type 2 diabetes (2, 4, 33, 34, 72, 76) and are positively correlated with insulin sensitivity (11, 16, 78), increased fatty acid oxidation in peripheral tissues (25, 66, 77), and protection of the liver from nonalcoholic fatty liver disease (7, 40, 57, 75) and fibrosis (32).

Endocannabinoids are lipid mediators that interact with G protein-coupled cannabinoid type 1 (CB1) and 2 (CB2) receptors to produce a wide range of biological effects similar to those of marijuana (50). Activation of CB1 results in increased food intake (20), insulin resistance (13, 45, 48, 60), hepatic lipogenesis (36, 49, 74), and reduced fatty acid β-oxidation (22, 35), which points to the important role of the endocannabinoid/CB1 system in obesity and its metabolic sequelae. Accordingly, chronic CB1 blockade or genetic knockout of CB1 results in decreased food intake, body weight, and adiposity, increased insulin and leptin sensitivity, and improvement in glucose and lipid homeostasis, hepatic steatosis, and fibrosis in rodent models of obesity (9, 17, 24, 26, 36, 37, 47, 54, 55, 58, 62–64), and similar effects were reported in obese subjects treated with rimonabant (12, 18, 52, 67, 69, 73). Chronic treatment with either globally acting or peripherally restricted CB1 antagonists has been shown to reverse the obesity-induced reduction in plasma adiponectin and adiponectin mRNA expression in adipose tissue (10, 27, 42, 62, 63, 65), suggesting a role for adiponectin in the improved metabolic homeostasis caused by CB1 blockade. To test this hypothesis, we examined the metabolic effects of CB1 blockade in Adipo−/− and Adipo+/- mice with high-fat diet (HFD)-induced obesity (DIO). The results indicate that the reversal of the HFD-induced hepatic steatosis and fibrosis by CB1 blockade requires intact adiponectin signaling; whereas the parallel reduction in body weight, improved hormonal homeostasis, and increased insulin sensitivity are independent of adiponectin.

MATERIALS AND METHODS

Animals. The experimental protocol used was approved by the Institutional Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health (NIH). Male 6-wk-old Adipo−/− mice and their wild-type littermate Adipo+/- controls were obtained from the Jackson Laboratory (Bar Harbor, ME). The mice were maintained under a 12:12-h light-dark cycle and fed ad libitum either a HFD (D12492; 60% of calories from fat, 20% from protein, and 20% from carbohydrates; Research Diets) or mouse standard diet (STD; NIH no. 31 rodent diet) for 7 mo.

Experimental protocol. HFD-fed obese Adipo−/− or Adipo+/- mice received vehicle (1% Tween 80, 4% DMSO, and 95% saline) or rimonabant, 10 mg/kg daily, for 7 days by intraperitoneal (ip) injection. Body weight was monitored daily. Mice were euthanized by cervical dislocation, the livers were removed and weighed, and samples of each liver were either snap-frozen or fixed in 10% buffered formalin. Trunk blood was collected for determining endocrine and...
biochemical parameters. Adiposity index was defined as the ratio of the combined weight of the epididymal, retroperitoneal, and subcutaneous fat pads to total body weight (63).

**Blood chemistry.** Blood was collected at the time the mice were euthanized. Serum alanine aminotransferases (ALT), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and triglycerides (TGs) were quantified using AMS Vegasys Chemistry Analyzer (Diamond Diagnostics). Blood glucose was determined using the Elite (Bayer) glucometer. Serum insulin was determined using the Ultra-Sensitive Mouse Insulin ELISA kit (Crystal Chem). Serum leptin and adiponectin were determined by ELISA (B-bridge International). Serum non-sterified free fatty acid (FFA) levels were measured using the HR Series NEFA kit (Wako Diagnostics).

**Hepatic TG content.** Liver tissue was extracted as described previously (23) and its TG content determined using EnzyChrom Tri-glyceride Assay Kit (Bioassay Systems).

Within 5 min, gastrocnemius muscle from hindlimbs and liver and renal cortex from C57BL/6 mice were anesthetized with intravenous injection of pentobarbital sodium. Venous blood samples were obtained from the inferior vena cava of HFD-induced obese or lean control Adipo−/− mice, as described previously (6) with modifications. Briefly, 5 days before the experiment, the left common carotid artery and the right jugular vein of HFD-induced obese or lean control Adipo−/− or Adipo+/+ mice were catheterized under isoflurane anesthesia. Following a 14-h period of fasting, clamps were performed on unrestrained, conscious mice treated with rimonabant (10 mg·kg−1·day−1·ip) or vehicle for 6 h days prior to the experiment. The clamp protocol consisted of a 120-min equilibration period (from t = −120 to 0 min), followed by a 120-min clamp period (from t = 0 to 120 min). A 5-μCi bolus of [3-3H]glucose (Perkin Elmer) was given at t = −120 min, followed by a 0.05 μCi/ml infusion for 2 h at a pump rate of 0.1 μl/min (CMA Microdialysis). The insulin clamp was begun at t = 0 min with a priming bolus (64 μl/kg) of human insulin (Humulin R; Eli Lilly), followed by an infusion (3.6 μl/kg·min−1) delivered at a pump rate of 0.1 μl/min from t = 0 to 120 min. The [3-3H]glucose infusion was increased to 0.1 μl/min for the remainder of the experiment. Specific activity for individual time points did not vary by >15% from the average specific activity during the last 40 min of the clamp. Euglycemia (~120–150 mg/dl) was maintained during clamps by measuring blood glucose every 10 min starting at t = 0 min and infusing 40% dextrose as necessary. Blood samples (60 μl) were taken every 10 min from t = 80 to 120 min and processed to determine glucose-specific activity. Mice received saline-washed erythrocytes from donors throughout the experimental period (4 μl/min) to prevent a fall of hematocrit by >5%. To estimate insulin-stimulated glucose fluxes in tissues, 2-deoxy-d-[1-14C]glucose (Perkin Elmer) was bolus administered (10 μCi) at t = 85 min, i.e., 45 min before the end of the experiment. At the end of the clamp, animals were anesthetized with intravenous injection of pentobarbital sodium. Within 5 min, gastrocnemius muscle from hindlimbs and liver and epididymal and subcutaneous fat were removed and frozen until analysis.

To determine [3-3H]glucose flux, plasma samples were deproteinized using barium hydroxide and zinc sulfate. The glucose production and disappearance rates were determined using Steele’s non-steady-state equations (61). Clamp hepatic endogenous glucose production rate was determined by subtracting the glucose infusion rate (GIR) from total glucose turnover (Ra). The glucose uptake by tissues and glycoen synthesis rates were calculated as described previously (81).

**Cell culture.** Human hepatoma HepG2 cells, purchased from the American Type Culture Collection, were plated in six-well plates at a density of 5 × 104 cells/ml and grown in Eagle’s Modified Essential Medium (EMEM) with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO2 at 37°C. Medium was changed every 2–3 days until cells reached 80–90% confluence. Prior to adiponectin treatment, cells were starved in serum-free EMEM for 24 h.

**Fatty acid uptake.** Palmitate uptake was initiated in HepG2 cells preincubated for 24 h with vehicle or adiponectin. Medium was replaced with an “incubation medium” containing serum-free EMEM supplemented with palmitic acid (final concentration: 500 μM) bound to fatty acid-free bovine serum albumin (BSA) at a 1:1 molar ratio and a trace amount (0.2 μCi) of radiolabeled [1-14C]palmitate (Perkin Elmer) at a specific activity of 2.220 MBq/nmol. Palmitate uptake was terminated after 1 h by removing the medium and washing twice with ice-cold PBS containing 0.5 mM MgCl2, 0.92 mM CaCl2, and 0.1% BSA. Then, cells were lysed with 1 M NaOH and centrifuged, and the supernatant was collected. [14C]palmitate was determined in the supernatant by liquid scintillation spectrometry (Beckman) and normalized for protein concentration. Results are reported as means ± SE of six samples/treatment from five independent experiments.

**Real-time PCR.** Total mRNA was extracted using Trizol (for mouse liver; Invitrogen) or RNasey Mini kit (for HepG2 cells; Qiagen), followed by DNase I treatment (Invitrogen), and reverse-transcribed using the iScript cDNA kit (Bio-Rad). Real-time PCR was performed using the StepOnePlus Real-time PCR system (Life Technologies) and the QuantiTect primer assays QT00291151, QT00149240, QT00137984, QT00106820, QT00162204, QT00155516, QT00135758, QT00140119, QT00096282, and QT01058253 against mouse Scd1, Fasn, Ppara, Cpt1a, Col1α1, Col3α1, Fln1, Acta2 (α-SMA), Timp1, and Cd36, respectively, and the QuantiTect Primer Assays QT00029181 against human Cd36. Normalization was performed with the QuantiTect primer assay against mouse 18S RNA (QT00164675) or human TATA box-binding protein (QT00000721).

**Western blot analyses.** Whole cell or liver extracts were prepared by using RIPA buffer (Thermo Fisher Scientific) containing protein inhibitor cocktail (Roche), and Western blotting was performed as described previously (62). Antibodies specific for CD36 and β-actin (Abcam) were used to detect the corresponding proteins.

**Materials.** Rimonabant was obtained from the National Institute of Drug Abuse Drug Supply Program. Human recombinant adiponectin was purchased from Enzo Life Sciences.

**Statistics.** Values are expressed as mean ± SE. Unpaired two-tailed Student’s t-test was used to determine differences between vehicle- and drug-treated groups. Time-dependent variables were analyzed, and results in multiple groups were compared by ANOVA followed by Bonferroni test. Significance was at P < 0.05.

**RESULTS**

Effects of rimonabant treatment on the metabolic profile of DIO mice. To examine the involvement of adiponectin in the improved metabolic profile by CB1 blockade, we tested the metabolic effects of short-term (7-day) treatment with 10 mg·kg−1·day−1 of rimonabant or vehicle in Adipo−/− and Adipo+/+ mice maintained on HFD for 7 mo, with age-matched lean controls providing baseline reference values.
Preliminary experiments indicated that 10 mg/kg was the maximally effective dose of rimonabant in improving the metabolic parameters measured, and treatment for 7 days achieved ~90% of the plateau response achieved by treatment for ~2 wk. HFD feeding induced similar weight gain and adiposity in the two strains, and the reversal of these parameters by rimonabant was also similar (Fig. 1, A and B). In parallel, rimonabant treatment significantly reduced serum cholesterol, TG, FFA, and leptin levels and increased the HDL/LDL cholesterol ratio irrespective of the adiponectin genotype (Fig. 2, A–E). Adiponectin was undetectable in serum of Adipo<sup>−/−</sup> mice and, consistent with previous reports (10, 27, 42, 62, 63, 65), serum adiponectin levels were reduced by the HFD, and the reduction was reversed by rimonabant in Adipo<sup>+/+</sup> mice (Fig. 2F).

Effects of CB<sub>1</sub> antagonism on glycemic control. Fasting blood glucose and serum insulin levels were markedly increased by HFD feeding and were similarly reduced by rimonabant in the two strains (Fig. 3, A and B). In response to a glucose challenge, both Adipo<sup>−/−</sup> and Adipo<sup>+/+</sup> mice on HFD showed a similar impairment of glucose tolerance, which was completely reversed by rimonabant treatment in both strains (Fig. 3, C and D). Similarly, rimonabant treatment improved insulin sensitivity to the same extent in the two strains (Fig. 3, E and F).

To analyze tissue-specific changes in insulin sensitivity, we performed hyperinsulinemic euglycemic clamp experiments in obese Adipo<sup>−/−</sup> and Adipo<sup>+/+</sup> mice treated with rimonabant and in respective lean controls. Preclamp basal plasma glucose levels were higher, and glucose infusion rates during the clamp were correspondingly lower in HFD-fed than in lean control animals, and rimonabant treatment of HFD-fed mice of both strains normalized these parameters (Fig. 4, A–D). The insulin-induced suppression of hepatic glucose production was significantly attenuated by HFD, and rimonabant treatment restored this effect of insulin in both strains (hepatic glucose production; Fig. 4E), resulting in increased GIR (Fig. 4F). On the other hand, CB<sub>1</sub> blockade was similarly ineffective in the two strains in altering whole body glucose clearance (R<sub>d</sub>; Fig. 4G), glucose uptake (Fig. 4H), and glycogen synthesis by skeletal muscle (Fig. 4I) or glucose uptake by subcutaneous or visceral fat tissue (Fig. 4, J and K). These observations indicate that the primarily hepatic form of insulin resistance induced by HFD and its reversal by short-term CB<sub>1</sub> blockade are independent of adiponectin.

Effect of rimonabant treatment on HFD-induced hepatic steatosis and fibrosis. We next analyzed the effect of rimonabant treatment on hepatic lipid parameters in Adipo<sup>−/−</sup> and Adipo<sup>+/+</sup> mice with DIO. The HFD-induced fatty liver, as visualized by histology and reflected in the elevated hepatic TG content, as well as the associated hepatocellular damage indicated by increased plasma ALT levels were significantly reduced by rimonabant only in Adipo<sup>+/+</sup> and not in Adipo<sup>−/−</sup> mice (Fig. 5, A–C). Similarly, rimonabant treatment increased the hepatic expression of lipid-mobilizing genes such as peroxisome proliferator-activated receptor-<i>α</i> (Ppara) and carnitine palmitoyl transferase-1 (Cpt-1) only in Adipo<sup>+/+</sup> and not in Adipo<sup>−/−</sup> mice (Fig. 5, D and E). In contrast, the expression of the lipogenic genes fatty acid synthase (Fasn) and stearoyl coenzyme-A desaturase 1 (Scd1) was similarly reduced by rimonabant in the two strains (Fig. 5, F and G).

In general, short-term HFD feeding (<14 wk) does not produce hepatic fibrosis (3), whereas long-term HFD feeding (28 wk or longer) may initiate it (70). Therefore, we evaluated the effect of rimonabant on hepatic fibrosis in Adipo<sup>−/−</sup> and Adipo<sup>+/+</sup> mice maintained on HFD for 7 mo. Hepatic collagen deposition was visualized using Sirius red (Fig. 6A) and quantified using digital image analysis (Fig. 6B). Significant collagen staining was present in Adipo<sup>−/−</sup> and Adipo<sup>+/+</sup> mice on HFD compared with STD-fed animals from the same strain. Similarly to its effect on hepatic steatosis, rimonabant treatment reduced collagen deposition in the liver only in Adipo<sup>+/+</sup> and not in Adipo<sup>−/−</sup> mice (Fig. 6, A and B). Consistent with these findings, the mRNA expression of the fibrosis markers <i>Pro-collagen</i>, <i>Coll1a1</i>, <i>Coll3a1</i>, <i>Fln1</i>, α-SMA, and <i>Timp1</i> was markedly increased in the liver of HFD-fed Adipo<sup>+/+</sup> mice compared with their lean controls, and rimonabant treatment significantly attenuated these increases, whereas it had no such effect in Adipo<sup>−/−</sup> mice (Fig. 6, C–H).

<i>Adiponectin regulates hepatic FFA uptake.</i> In addition to decreased fatty acid β-oxidation, HFD-induced steatosis also involves increased fatty acid uptake (28). Therefore, we explored the role of adiponectin and CB<sub>1</sub> receptors in regulating hepatic fatty acid uptake. When compared with STD-fed lean animals, both HFD-fed Adipo<sup>−/−</sup> and Adipo<sup>+/+</sup> mice showed a dramatic increase in the hepatic mRNA and protein levels of...
fatty acid translocase/CD36 (Fig. 7, A and B), a protein that mediates the uptake of FFA from the circulation to the liver (31), whereas rimonabant treatment reversed this increase only in Adipo−/− and not in Adipo+/+ mice.

Additionally, we analyzed hepatic FFA uptake directly by measuring the uptake of [14C]palmitate into human hepatoma HepG2 cells, a suitable model for FFA uptake by hepatocytes (53), along with CD36 mRNA and protein levels. Exposure of HepG2 cells to increasing concentrations (0.1–5 μg/ml) of human recombinant adiponectin decreased CD36 mRNA and protein levels in a concentration-dependent manner (Fig. 7, C and D), with parallel reductions in the incorporation of [14C]palmitate into the cells (Fig. 7E).

**DISCUSSION**

The significant role of endocannabinoids acting through CB1 in appetite regulation, energy balance, and metabolic homeostasis is well established (50). Chronic blockade of CB1 has been shown to improve cardiometabolic risk associated with visceral obesity and its metabolic complications, and this was associated with the reversal of the obesity-related reduction in plasma adiponectin levels in both animals (62, 63) and humans (18, 19, 56, 68). In the present study, which was designed to explore the role of adiponectin in mediating the metabolic effects of CB1 antagonism in obesity, we demonstrate that the reversal of HFD-induced hepatic steatosis and fibrosis by CB1...
blockade is adiponectin dependent and involves both reduced uptake and increased β-oxidation of FFA in the liver. On the other hand, the effects of CB₁ blockade in reducing body weight and fat mass, improving hormonal and glucose metabolism, and increasing insulin sensitivity occur independently of adiponectin.

The HFD-induced accumulation of TGs in the liver is likely the result of the transfer of FFAs from adipose tissue (37, 44), with increased activity of hepatic CB₁ playing only a minor role (45). On the other hand, global or peripheral CB₁ blockade inhibits hepatic lipogenic gene expression and de novo lipogenesis, whereas it increases β-oxidation of FFAs, resulting in a complete reversal of obesity-induced fatty liver (36, 37, 49, 59, 63). Adiponectin, acting via its hepatic type 2 receptor (AdipoR2), also protects the liver from the HFD-induced TG accumulation, increased inflammation, and reduced fatty acid β-oxidation (7, 40, 57, 75, 77, 80). Indeed, the present findings indicate that chronic CB₁ blockade reduces HFD-induced steatosis and fibrosis as well as hepatocellular injury by an adiponectin-dependent mechanism, as indicated by the absence of these effects in Adipo⁻/⁻ mice (Figs. 5 and 6), despite the same degree of weight loss, reduced adiposity, and increased insulin sensitivity as in rimonabant-treated Adipo⁺/+ mice.

The decreased gene expression of the hepatic lipogenic enzymes Fasn and Scd1 by rimonabant was also similar in the two strains (Fig. 5, F and G). This indicates not only that these effects are independent of adiponectin but also that they do not contribute significantly to the alleviation of hepatic steatosis by rimonabant treatment. On the other hand, rimonabant treatment increased fatty acid β-oxidation only in Adipo⁺/+ mice, as documented by the increased gene expression of Pparα and its target Cpt-1, the rate-limiting enzyme in fatty acid oxidation (Fig. 5, D and E). This indicates a requirement for adiponectin in the antisteatotic effect of chronic CB₁ blockade in DIO, which is also consistent with the well-established hepatoprotective function of adiponectin via stimulation of PPARα activity (77, 78, 80). A similar requirement for adiponectin in the antisteatotic effect of CB₁ blockade has...
also been noted in a rare form of obesity due to the absence of leptin (71) and has been linked in humans to a polymorphism (G1359A) in the gene encoding the CB1 receptor (1).

An additional mechanism involved in the hepatoprotective effect of adiponectin is inhibition of FFA uptake into hepatocytes, as documented by the reduced uptake of palmitate (Fig. 7E), and a parallel reduction in CD36 expression in HepG2 cells exposed to adiponectin (Fig. 7, C and D). These findings are compatible with in vivo data on CD36 expression in the liver, which confirm the well-established upregulation of hepatic CD36 by HFD (28,
and also demonstrate that this effect of HFD present in both strains is reversed by rimonabant only in wild-type and not in Adipo<sup>−/−</sup> mice (Fig. 7, A and B). A similar reduction in the hepatic expression of CD36 has been demonstrated recently in DIO mice treated with the peripherally restricted CB<sub>1</sub> antagonist JD 5037 (15), which also reverses the HFD-induced hepatic steatosis and reduction in adiponectin levels (62). Taken together, our results suggest that the antisteatotic effect of CB<sub>1</sub> blockade is mediated indirectly by adiponectin, which acts directly on hepatocytes to reduce FFA uptake and increase fatty acid β-oxidation.
The antifibrogenic effect of adiponectin is also well documented in rodents (5, 32, 38, 39), and reduced serum adiponectin levels are associated with liver fibrosis in humans (8, 57). Long-term HFD feeding resulted in increased gene expression of fibrotic markers, with histological evidence of fibrosis in both Adipo+/+ and Adipo−/− mice (Fig. 6). There is evidence that endocannabinoids acting via hepatic CB1 exert profibrotic effects in the liver and that chronic CB1 blockade or knockdown ameliorates hepatic fibrosis (14, 17, 30, 64), but the underlying mechanisms are unclear. The ability of rimonabant to reverse HFD-induced fibrosis in wild-type but not in adiponectin-deficient mice, as documented here, clearly indicates the obligatory role of adiponectin in mediating the antifibrotic effect of CB1 blockade. Furthermore, the antifibrotic effect of

Fig. 6. Rimo reduces the long-term HFD-induced hepatic fibrosis in Adipo+/+ but not Adipo−/− mice. Obese Adipo−/− and Adipo+/+ mice were treated with 10 mg·kg⁻¹·day⁻¹ ip of Rimo or Veh for 7 days. Collagen deposition was evaluated by Sirius red staining (A) and quantified as described in MATERIALS AND METHODS (B). Note the reduced deposition of collagen fibers in the liver of Adipo+/+ mice treated with Rimo as well as the normalized mRNA expression levels of the profibrogenic markers: pro-collagen (C), Col1α1 (D), Col3α1 (E), Fn1 (F), α-SMA (G), and Timp1 (H). Data represent means ± SE from 4 to 5 mice/group. *P < 0.05 relative to the corresponding values in STD-Veh group; #P < 0.05 relative to corresponding values in HFD-Veh group.
rimonabant can be dissociated from the parallel reduction in body weight and the improved insulin sensitivity, which are present in both strains. Further studies should address whether the profibrotic effect of endocannabinoids/hepatic CB1 is due to reduced adiponectin signaling in the liver.

Compelling evidence indicates that adiponectin is a major insulin-sensitizing adipokine (11, 16, 72, 78). Chronic CB1 blockade, which alleviates obesity-induced insulin resistance via both central and peripheral mechanisms (13, 42, 45, 47, 48, 62, 63), also normalizes the reduced plasma levels of adiponectin in obese animals and humans (10, 18, 19, 27, 29, 42, 62, 63, 65), which could suggest adiponectin involvement in the reversal of insulin resistance by CB1 blockade. However, this is not supported by the present findings, which indicate that the insulin-sensitizing effect of rimonabant is similar in the presence or absence of adiponectin, as indicated by a number of parameters. Thus, the effects of rimonabant were similar in obese Adipo⁻/⁻ or Adipo⁺/⁺ mice in reversing the fasting hyperglycemia and hyperinsulinemia (Fig. 3, A and B) and normalizing the glucose intolerance and insulin resistance, as tested by glucose tolerance test and insulin sensitivity test (Fig. 3, C–F). The effects of rimonabant on tissue-specific insulin sensitivity were also similar, as tested using a hyperinsulinemic euglycemic clamp. Namely, rimonabant treatment reversed the marked hepatic insulin resistance without significantly altering glucose uptake into muscle and fat in both strains. These findings are compatible with rimonabant directly targeting hepatocyte CB1, the activation of which has been shown to induce hepatic insulin resistance by inhibiting insulin signaling via insulin receptor substrate-1 and Akt-2 and causing a parallel reduction in insulin clearance via downregulating the insulin-degrading enzyme (45). Therefore, the increase in plasma adiponectin by short-term rimonabant treatment is not causally related to the parallel improvement of insulin resistance and may be a consequence of the improved metabolic profile.

In agreement with the present findings, the reduction of body weight and adiposity by CB1 antagonist treatment was found to be independent of adiponectin in leptin-deficient ob/ob mice (71) and in DIO mice (46). Similar to the DIO mice tested in the present study, the insulin resistance of ob/ob mice was due primarily to hepatic insulin resistance, which was improved by rimonabant in both the presence and absence of adiponectin. However, the improvement caused by rimonabant treatment was significantly less in the absence than in the presence of adiponectin, indicating an additional adiponectin-dependent mechanism (71). The presence of such a component in ob/ob but not in DIO mice may be related to the different obesity models (genetic vs. diet-induced) or the different duration of rimonabant treatment (3 vs. 1 wk).

The present findings also differ from the results of Migrenne et al. (46), who used a DIO mouse model similar to that in the present study but found that rimonabant treatment for 4 wk resulted in decreased hepatic glucose production and increased
glucose utilization in adipose tissue during an insulin clamp in Adipo<sup>++</sup> mice, whereas no such effects were observed in Adipo<sup>−/−</sup> mice, which suggested an adiponectin-dependent mechanism. There are a number of factors that may account for the discrepant results. The DIO mice in the earlier study had a body weight of &lt;40 g (46) compared with the ~50 g of body weight in the present study. It has been shown that, despite being an inbred strain, C57Bl6 mice maintained on a HFD have a clearly bimodal body weight distribution, with the low (&lt;40 g) and high body weight groups (45–55 g) also displaying qualitative differences in their metabolic profile (21, 51). It is possible that the requirement for adiponectin for the glycemic response to CB1 blockade is limited to mice that are partially resistant to HFD-induced obesity. It is also possible that an adiponectin-dependent component in the insulin-sensitizing effect of CB1 blockade emerges only following more prolonged treatment, such as that used in the earlier study. However, in the same study, rimonabant was able to completely reverse the baseline hyperinsulinemia in both Adipo<sup>++</sup> and Adipo<sup>−/−</sup> mice (46), which complicates interpretation of these findings as it argues against adiponectin involvement in the improved insulin sensitivity.

In summary, the present findings indicate a differential role of adiponectin in the beneficial metabolic effects of CB<sub>1</sub> antagonism in obesity/metabolic syndrome. Namely, adiponectin appears to mediate the reversal of hepatic steatosis and fibrosis by CB<sub>1</sub> antagonism through reducing FFA uptake into the liver and inducing a PPAR<sub>α/CPT-1</sub>-mediated increase in hepatic fatty acid oxidation, whereas the reduction in body weight and adiposity and improved glucose and insulin homeostasis resulting from short-term CB<sub>1</sub> blockade are independent from adiponectin.

ACKNOWLEDGMENTS
Address for correspondence for J. Tam after June 2014: Obesity and Metabolism Laboratory, School of Pharmacy, Institute for Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, 91120, Israel (e-mail: yossit@ekmd.huji.ac.il).

GRANTS
This work was supported by intramural funds from the National Institute on Alcohol Abuse and Alcoholism, NIH.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
J.T. and G.K. contributed to the conception and design of the research; J.T., G.G., B.J.E., and L.Z. performed the experiments; J.T., G.G., and B.J.E. analyzed the data; J.T., G.G., B.J.E., T.J., G.S., R.C., and G.K. interpreted the results of the experiments; J.T. prepared the figures; J.T. drafted the manuscript; J.T. and G.K. edited and revised the manuscript; J.T., G.G., L.Z., T.J., G.S., and G.K. approved the final version of the manuscript.

REFERENCES
ROLE OF ADIPOGENIC IN CB1 BLOCKADE


