ORIGINAL ARTICLE

Basic Study

Metabolic and inflammatory functions of cannabinoid receptor type 1 are differentially modulated by adiponectin

Qiong Wei, Jong Han Lee, Chia-Shan Wu, Qun S Zang, Shaodong Guo, Hui-Chen Lu, Yuxiang Sun

ORCID number: Qiong Wei 0000-0001-6836-9929; Jong Han Lee 0000-0002-5614-1537; Chia-Shan Wu 0000-0002-6034-939X; Qun S Zang 0000-0002-6397-8896; Shaodong Guo 0000-0001-5126-731X; Hui-Chen Lu 0000-0002-6628-7177; Yuxiang Sun 0000-0002-6656-3733.

Author contributions: Wei Q, Lee JH and Wu CS performed the experiments, analyzed the data and wrote the paper; Guo S and Zang QS consulted the study and proofread the paper; Lu HC provided the *CB1* knockout mice, consulted in the study and proofread the paper; Sun Y designed the study and wrote the paper; all authors wrote, read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board at Baylor College of Medicine.

Institutional animal care and use committee statement: All animal experiments conformed to the internationally accepted principles for the care and use of laboratory animals (Protocol AN-2770, The

Qiong Wei, Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing 210009, Jiangsu Province, China

Qiong Wei, Jong Han Lee, Chia-Shan Wu, Yuxiang Sun, Department of Pediatrics, USDA/ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030, United States

Jong Han Lee, Department of Marine Bioindustry, Hanseo University, Seosan 31962, South Korea

Chia-Shan Wu, Shaodong Guo, Yuxiang Sun, Department of Nutrition, Texas A and M University, College Station, TX 7743, United States

Qun S Zang, Department of Surgery, Stritch School of Medicine, Loyola University Chicago Health Science Campus, Maywood, IL 60153, United States

Hui-Chen Lu, Department of Psychological and Brain Sciences, Linda and Jack Gill Center of for Biomolecular Science, Bloomington, IN 47405, United States

Corresponding author: Yuxiang Sun, MD, PhD, Associate Professor, Department of Nutrition, Texas A and M University, 214C Cater-Mattil, 2253 TAMU College Station, TX 7743, United States. yuxiang.sun@tamu.edu

Abstract

BACKGROUND
Antagonists of cannabinoid type 1 receptor (*CB1*) have been shown to promote body weight loss and improve insulin sensitivity. Cannabinoids decrease adiponectin, and *CB1* blocker increase adiponectin. However, the mediators of *CB1* actions are not well defined.

AIM
To investigate whether the beneficial effects of *CB1* inhibition are, at least in part, mediated by adiponectin.

METHODS
We compared metabolic and inflammatory phenotypes of wild-type (WT) mice, *CB1*-null (*CB1^{-/-}*) and *CB1*/adiponectin double-knockout (DKO) mice. We assessed the insulin sensitivity using insulin tolerance test and glucose tolerance test, and inflammation using flow cytometry analysis of macrophages.

RESULTS

CB1$^{-/-}$ mice exhibited significantly reduced body weight and fat mass when compared to WT mice. While no significance was found in total daily food intake and locomotor activity, CB1$^{-/-}$ mice showed increased energy expenditure, enhanced thermogenesis in brown adipose tissue (BAT), and improved insulin sensitivity compared to WT mice. DKO showed no difference in body weight, adiposity, nor insulin sensitivity; only showed a modestly elevated thermogenesis in BAT compared to CB1$^{-/-}$ mice. The metabolic phenotype of DKO is largely similar to CB1$^{-/-}$ mice, suggesting that adiponectin is not a key mediator of the metabolic effects of CB1. Interestingly, CB1$^{-/-}$ mice showed reduced pro-inflammatory macrophage polarization in both peritoneal macrophages and adipose tissue macrophages compared to WT mice; in contrast, DKO mice exhibited increased pro-inflammatory macrophage polarization in these macrophages compared to CB1$^{-/-}$ mice, suggesting that adiponectin is an important mediator of the inflammatory effect of CB1.

CONCLUSION

Our findings reveal that CB1 functions through both adiponectin-dependent and adiponectin-independent mechanisms: CB1 regulates energy metabolism in an adiponectin-independent manner, and inflammation in an adiponectin-dependent manner. The differential effects of adiponectin on CB1-mediated metabolic and inflammatory functions should be taken into consideration in CB1 antagonist utilization.

Key Words: Cannabinoid type 1 receptor; Adiponectin; Thermogenesis; Macrophages; Inflammation; Insulin resistance

Core Tip: Antagonists of cannabinoid type 1 receptor (CB1) have been shown to promote body weight loss and improve insulin sensitivity. Cannabinoids have been shown to regulate adiponectin. However, it is unclear whether adiponectin is a key mediator of the functions of CB1. We compared metabolic and inflammatory phenotypes of CB1-null *vs* CB1/adiponectin double-knockout mice. Our findings reveal that CB1 functions through both adiponectin-dependent and adiponectin-independent mechanisms: CB1 regulates energy metabolism in an adiponectin-independent manner, and inflammation in an adiponectin-dependent manner.

INTRODUCTION

The incidence of obesity has increased rapidly during recent decades, particularly in developed/industrialized countries. Obesity increases the incidences of hyperinsulinemia, insulin resistance, type 2 diabetes, dyslipidemia, atherosclerosis, hypertension, inflammation, and cancer[1,2]. Endocannabinoids are key regulators of food intake and energy metabolism, and the effects are mediated through the activation of the cannabinoid type 1 receptor 1 (CB1)[3,4]. Recent studies have demonstrated that blocking the activity of the endogenous cannabinoid system might be a strategy for the treatment of obesity and metabolic syndrome[5-7].

Previous study demonstrated that CB1 knockout mice consume less food and have reduced body weight[4,8]. Rimonabant, a specific antagonist of CB1, reduces food intake by blocking the orexigenic effect of cannabinoids[9]. There is also evidence that endogenous cannabinoids regulate energy expenditure[10]. It has been shown that

Prysyazhnyuk V
S-Editor: Wang LL
L-Editor: A
P-Editor: Yu HG

virally-induced hypothalamic *CB1* knockout mice showed no change in food intake, but did show less body weight gain over time due to increased energy expenditure; and the mRNA expression of β3-adrenergic receptor and uncoupling protein-1 (UCP-1) was elevated in the brown adipose tissue (BAT)[10]. There are also data showing that rimonabant alleviates dyslipidemia and obesity *via* a BAT thermogenesis-mediated increase of energy expenditure[9]. It has been shown that peripheral *CB1* blockade is effective in activating thermogenesis in BAT to mitigate dyslipidemia and obesity[11], which suggests that the function of *CB1* in BAT can be peripherally mediated and is not necessarily dependent on its central action. These results suggest that endocannabinoids may regulate energy metabolism by binding to *CB1* expressing cells in peripheral white adipose tissue (WAT)[8] and/or BAT[9].

Adiponectin, an adipokine with insulin-sensitizing functions, has been reported to be relevant in many metabolic diseases such as obesity, and with associated complications such as diabetes, hyperinsulinemia, insulin resistance, dyslipidemia, hypertension, and inflammation[12]. Adiponectin treatment reduces body weight, improves hyperglycemia, ameliorates hyperinsulinemia and insulin resistance, and increases fatty acid oxidation and lipid clearance, in animal models of obesity and diabetes[13,14]. One of the most intriguing consequences of rimonabant treatment is increased adiponectin gene expression in adipose tissue of diet-induced obese (DIO) mice[9] and in cultured adipocytes[15]. However, the rimonabant-treated adiponectin- and leptin-deficient mice exhibit significantly ameliorated insulin resistance, which suggests that rimonabant reduces insulin resistance *via* both adiponectin-dependent and adiponectin-independent mechanisms[16]. These results suggest that rimonabant may regulate adiponectin expression in adipocytes, and the metabolic effects of rimonabant, at least in part, could be due to enhanced adiponectin secretion.

To determine whether adiponectin is indeed required for the peripheral functions of *CB1*, we used a genetic approach by breeding *CB1-/-* mice with adiponectin-deficient mice to generate a mouse model lacking both *CB1* and adiponectin, *aka* double KO (DKO). We studied metabolic regulation such as thermogenesis and insulin sensitivity in these mice. The link between inflammation and obesity is now increasingly recognized and inflammation is considered a culprit of insulin resistance. Thus, we also characterized macrophage polarization in peritoneal macrophages and adipose tissue macrophages to elucidate whether *CB1* acts through adiponectin to modulate CB-1 mediated inflammation.

MATERIALS AND METHODS

Animals

All procedures using animal experiments were approved by the Institution of Animal Care and Use Committee at Baylor College of Medicine. All mice used in this study were congenic male mice. All mice were on a pure C57/6J background. To generate mice lacking both *CB1* and adiponectin, *CB1-/-* mice and *adiponectin-/-* mice were bred to each other to create compound heterozygotes that were *CB1+/-/adiponectin+/-*. In the second cross, compound heterozygotes were further bred to each other to yield homozygous *CB1-/-/adiponectin-/-* (*aka* double-knockout DKO mice); *CB1-/-* adiponectin+/+ mice (aka *CB1-/-*), and *CB1+/+* adiponectin+/+ (aka WT mice). Age-matched male WT, *CB1-/-* and DKO were used in the studies. There were three groups of mice used in the study: (WT) control group, *CB1-/-* group, DKO group. Animals were housed under controlled temperature and lighting (75 ± 1 ℉; 12 h light-dark cycle). The diet was from Harlan-Teklad (2920X) and the diet compositions are as follows: 16% of calories from fat, 60% from carbohydrates, and 24% from protein. All experiments were approved by the Animal Care Research Committee of the Baylor College of Medicine.

Metabolic characterizations

Magnetic Resonance Imaging analysis of body composition was also carried out using an EchoMRI Whole Body Composition Analyzer (Echo MRI®, United States). Metabolic parameters were obtained using an Oxymax open-circuit indirect calorimetry Comprehensive Lab Animal Monitoring System (CLAMS) from Columbus Instruments (Columbus, OH, United States). Energy expenditure (EE) was calculated as the product of the value of oxygen (3.815 + 1.232 × RQ) and the volume of O_2 consumed. Respiratory quotient (RQ) ratio of VCO_2/VO_2 was then calculated[17]. Energy expenditure was normalized to both body weight and lean mass. Locomotor activity was measured using infrared beams to count the number of beam breaks

during the recording period. The CLAMS data was the average of 3 d of data that were collected after 3 d of acclimation.

Insulin tolerance test and glucose tolerance test

The Insulin tolerance test (ITT) and glucose tolerance test (GTT) were carried out on WT, *CB1⁻/⁻* and DKO mice. For ITT, after being fasted for 6 h, glucose of mouse tail blood was measured using One Touch Ultra glucose meter (lifeScan, New Brunswick, NJ, United States). It can detect glucose concentrations from 20 to 600 mg/dl using an electrochemical biosensor technology based on glucose oxidase chemistry. Mice then received an *i.p.* injection of human insulin (Eli Lilly Indianapolis, IN, United States) at a dose of 1.0 U kg-1 of body weight. Tail blood glucose concentration was measured at 0, 30, 60, 90 and 120 min after *i.p.* insulin injection. The GTT was carried out after the mice were fasted for 18 h overnight. The mice received *i.p.* injection of glucose (Sigma-Aldrich, St. Louis, MO, United States) at a dose of 2.0 g kg-1 body weight. The tail blood glucose was measured at 0, 15, 30, 60 and 120 min after glucose injection, and blood was collected for ELISA insulin analysis at 0, 15, 30 and 120 min after glucose injection.

Flow cytometry analysis

Peritoneal macrophage and stromal vascular (SV) cells of epididymal adipose tissues were fractionated as described[18,19]. Briefly, to get peritoneal macrophage, 5 ml of cold phosphate buffer saline (PBS) was injected into mouse peritoneal cavities immediately after anesthesia. After shaking the mice for 2-3 min, peritoneal fluid was harvested and spun down for peritoneal macrophages at 500 g for 5 min at 4 °C. The stromal vascular cells were isolated from the equal mass of epididymal adipose tissues using the collagenase digestion method. For flow cytometry analysis, same quantity cells (1×10^6) were subsequently re-suspended and stained with appropriate antibodies (F4/80 and CD11c for M1 type macrophage, or F4/80 and CD206 for M2 type macrophage) as described in our previous study[20]. Antibody information used in flow cytometry analysis is as follows: PE anti-mouse F4/80 antigen (eBioscience, San Diego, CA), FITC anti-mouse CD11c antigen (BD Bioscience, San Jose, CA), purified CD16/CD32 antigen (BD Bioscience, San Jose, CA), and APC anti-mouse CD206 antigen (BD Bioscience, San Jose, CA). All data were collected using FACScan and analyzed using CellQuest software (BD Biosciences, San Jose, CA).

Analysis of gene expression

BAT and WAT were snap-frozen in liquid nitrogen and stored at -80 °C. Total RNA was extracted from frozen tissue samples using TRIzol Reagent (Invitrogen, Carlsbad, CA). RNA was subsequently treated with DNase (Ambion, Austin, TX). RNA quality was assessed on 1.5% agarose gel electrophoresis in the presence of formaldehyde, and RNA concentration was determined by NanoDrop. The cDNA was synthesized from 1g RNA using the Superscript III First-Strand Synthesis system for reverse transcription-polymerase chain reaction (RT-PCR) (Invitrogen). Quantitative real-time RT-PCR was performed on an ABI7900 using the SYBR Green PCR Master Mix or the Taqman gene expression Master Mix (Applied Biosystems, Carlsbad, CA, United States). After amplification, the PCR product was subjected to 2% agarose gel electro-phoresis. 18S RNA and -actin were used as internal controls. The primer sequences of quantitative RT-PCR are listed in Table 1 below.

Data analysis

Data are expressed as means ± SEM. Two groups were compared by *t*-test. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out with SPSS 23.0 statistical software (IBM, Armonk, NY, United States).

RESULTS

CB1 ablation increases energy expenditure, reduces adiposity, and improves insulin sensitivity

The body weights of *CB1⁻/⁻* mice were significantly lower than WT littermates; the analysis of body composition showed a markedly decreased percentage of fat mass in *CB1⁻/⁻* mice compared to WT mice (Figure 1A). We then assessed food intake, locomotor activity, and energy expenditure using CLAMS. Our data showed there was a trend of reduction but no significant difference in total daily food intake by *CB1⁻/⁻*

Table 1 The sequences of reverse transcription-polymerase chain reaction primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
UCP-1	GTGAAGGTCAGAATGCAAGC	AGGGCCCCCTTCATGAGGTC
PGC-1α	CATTTGATGCACTGACAGATGGA	CCGTCAGGCATGGAGGAA
IR	CAAAAGCACAATCAGAGTGAGTATGAC	ACCACGTTGTGCAGGTAATCC
IRS1	GCCTGGAGTATTATGAGAACGAGAA	GGGGATCGAGCGTTTGG
PPARγ2	GCCTATGAGCACTTCACAAGAAATT	TGCGAGTGGTCTTCCATCAC
GLUT4	GCCTTGGGAACACTCAACCA	CACCTGGGCAACCAGAATG
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
CD11C	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTC
CD206	TGATTACGAGCAGTGGAAGC	GTTCACCGTAAGCCCAATTT
TNFα	GAGAAAGTCAACCTCCTCTCTG	GAAGACTCCTCCCAGGTATATG
IL-1β	TGTTCTTTGAAGTTGACGGACCC	TCATCTCGGAGCCTGTAGTGC
IL-6	CCAGAGATACAAAGAAATGATGG	ACTCCAGAAGACCAGAGGAAAT

mice compared to WT mice (Figure 1B). To further determine whether there is a difference in locomotor activity, we analyzed spontaneous locomotor activity of these mice. Neither total daily locomotor activity nor the locomotor activity during light or dark cycles was altered (Figure 1C). We next calculated energy expenditure and found that energy expenditure of *CB1*^{-/-} mice was higher compared to their WT counterparts when normalized to body weight but no difference when normalized to lean mass (Figure 1D). Together, the results indicate that while *CB1* ablation reduces body weight and fat deposition, it may be a due to a combination of changes in food intake and exergy expenditure.

Next, we assessed the glycemic phenotype. ITT showed that *CB1*^{-/-} mice were more responsive to insulin challenge than WT mice (Figure 1E). During GTT, there was no difference in glucose clearance following a glucose load in WT and *CB1*^{-/-} mice; but remarkably, the insulin levels of *CB1*^{-/-} mice were significantly lower, indicative of increased insulin sensitivity (Figure 1F). These results indicate that *CB1*^{-/-} mice have improved insulin sensitivity, which is in line with reduced body weight and body fat.

Collectively, these data suggest that *CB1* is an important regulator of energy homeostasis and insulin sensitivity.

Adiponectin has little impact on CB1-mediated overall metabolic profile

To determine whether the metabolic effects of *CB1* are mediated by adiponectin, we compared the metabolic phenotypes of *CB1*^{-/-} and DKO mice. The body weights of *CB1*^{-/-} mice were similar to their age-matched DKO (Figure 2A). There were also no differences in fat mass and lean mass between *CB1*^{-/-} and DKO mice. Indirect calorimetry analysis showed similar total food intake (Figure 2B) and locomotor activity (Figure 2C) between DKO and *CB1*^{-/-} mice. Interestingly, compared to *CB1*^{-/-} mice, DKO mice had increased energy expenditure when corrected either by total body weight or by lean weight (Figure 2D).

Furthermore, there was no difference in insulin sensitivity assessment of ITT between *CB1*^{-/-} and DKO mice (Figure 2E). We further assessed glucose response during GTT: No difference was detected in glucose response, but interestingly, the insulin of DKO was lower at 15 min but higher at 120 min as compared to that of *CB1*^{-/-} mice (Figure 2F). These data suggest that DKO mice have mostly similar metabolic profile, insulin sensitivity and glycemic response as *CB1*^{-/-} mice, despite there are some varied insulin responses to glucose. Taken together, the effects of *CB1* on metabolism are dominant; adiponectin is not essential in mediating the metabolic effects of *CB1*.

CB1 ablation activates thermogenesis in BAT

To determine the underlying mechanisms of the increased energy expenditure observed in *CB1*^{-/-} mice, we subsequently analyzed BAT collected from the mice. *CB1*^{-/-} mice showed a decreased ratio of BAT: Body weight as compared to WT mice (Figure 3A). Mitochondrial uncoupling protein 1 (UCP1) is the hallmark regulator of mitochondrial biogenesis and thermogenesis; when activated, UCP1 dissipates the
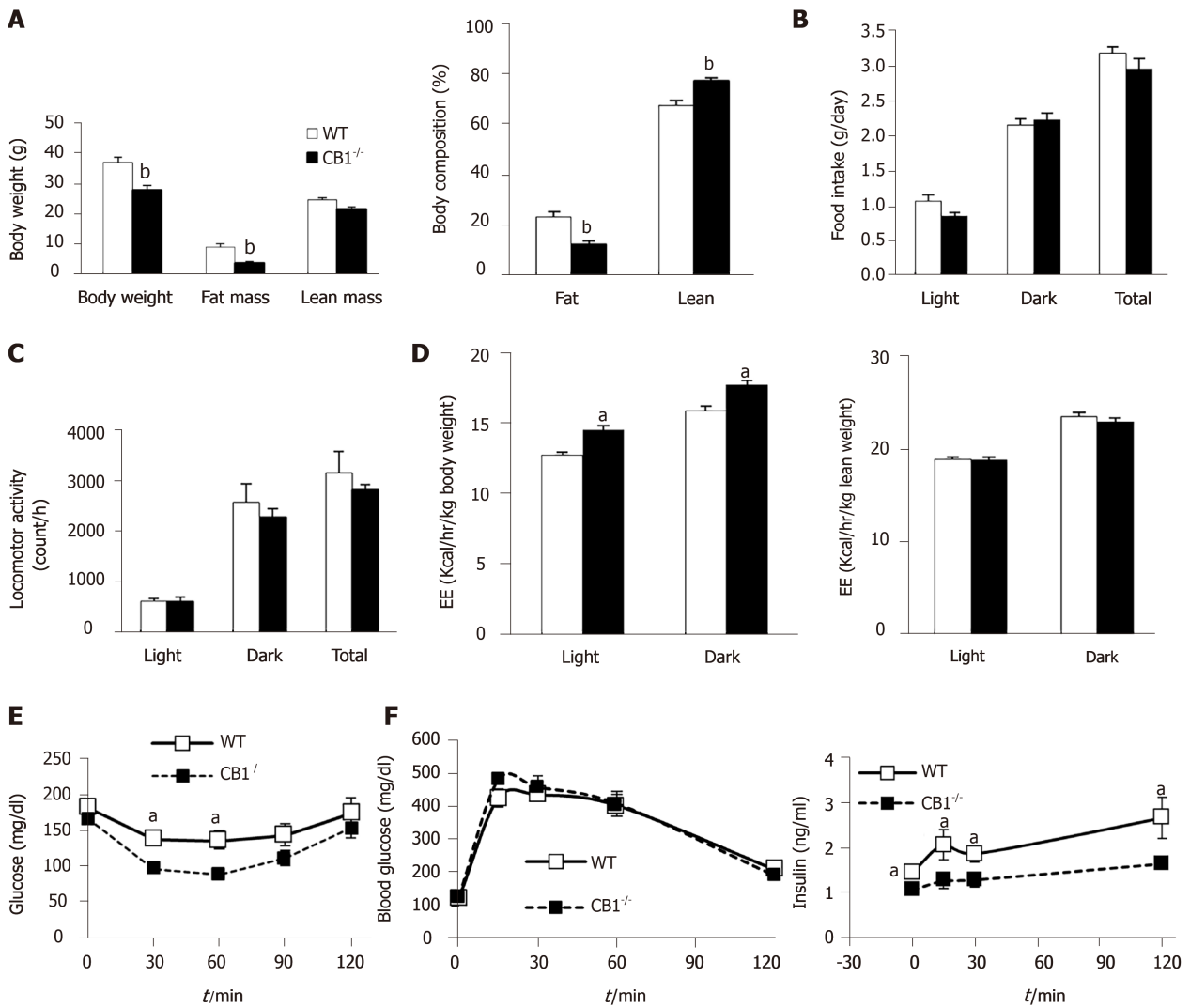
Figure 1 Cannabinoid type 1 receptor-null mice have reduced adiposity and improved insulin sensitivity. Wild-type (WT) and cannabinoid type 1 receptor-null (*CB1⁻/⁻*) male mice at 4 mo of age. A: Body weight and body composition; B: Daily food intake; C: Locomotor activity; D: Energy expenditure adjusted by body weight or lean mass; E: Insulin tolerance tests; F: Glucose tolerance tests at 5 mo of age. *n* = 5-7. $^aP < 0.05$, $^bP < 0.001$, WT *vs CB1⁻/⁻*.

transmembrane proton gradient and generates heat[21]. UCP1 mRNA was increased in *CB1⁻/⁻* mice as compared to WT controls (Figure 3B). Peroxisome proliferator-activated receptorγcoactivator-1 (PGC-1) is an upstream regulator of UCP1[22]. Indeed, PGC-1 expression was also increased in the *CB1⁻/⁻* mice when compared to that of WT mice (Figure 3B).

Our result in Figure 1 showed *CB1⁻/⁻* mice have higher insulin sensitivity compared to WT mice. Consistently, the gene expression of insulin receptor (IR) and insulin receptor substrate 1 (IRS-1) were increased in BAT of *CB1⁻/⁻* mice. Peroxisome proliferator-activated receptorsγ2 (PPARγ2) is an important master adipogenic regulator [11]. Here we found that PPARγ2 was higher in BAT of *CB1⁻/⁻* mice (Figure 3B). Glucose transporter type 4 (GLUT4) is a key mediator of glucose uptake in the adipose tissues[23]. As expected, GLUT4 expression in BAT of *CB1⁻/⁻* mice was increased (Figure 3B), supporting increased glucose uptake and consistent with increased heat production. Together, ablation of *CB1* increased BAT thermogenic activity, likely by modulating mitochondrial function, insulin signaling, adipogenesis, and glycose uptake signaling pathways in BAT.

We have reported that adiponectin has an important role in body temperature maintenance and thermogenesis. Here, we compared the weight of BAT depots in *CB1⁻/⁻* and DKO mice. There was also no difference in total weight or BAT percentage between *CB1⁻/⁻* and DKO mice (Figure 3C). The expression of thermogenic regulators UCP1 and PGC-1 was increased in BAT of DKO mice compared to that of *CB1⁻/⁻* mice, while the expression of IR and IRS-1, GLUT4, and PPARγ2 were unchanged (Figure 3D). These results suggest that while adiponectin may be an important mediator for the effect of *CB1* on mitochondrial genes in BAT, it is not critical for the
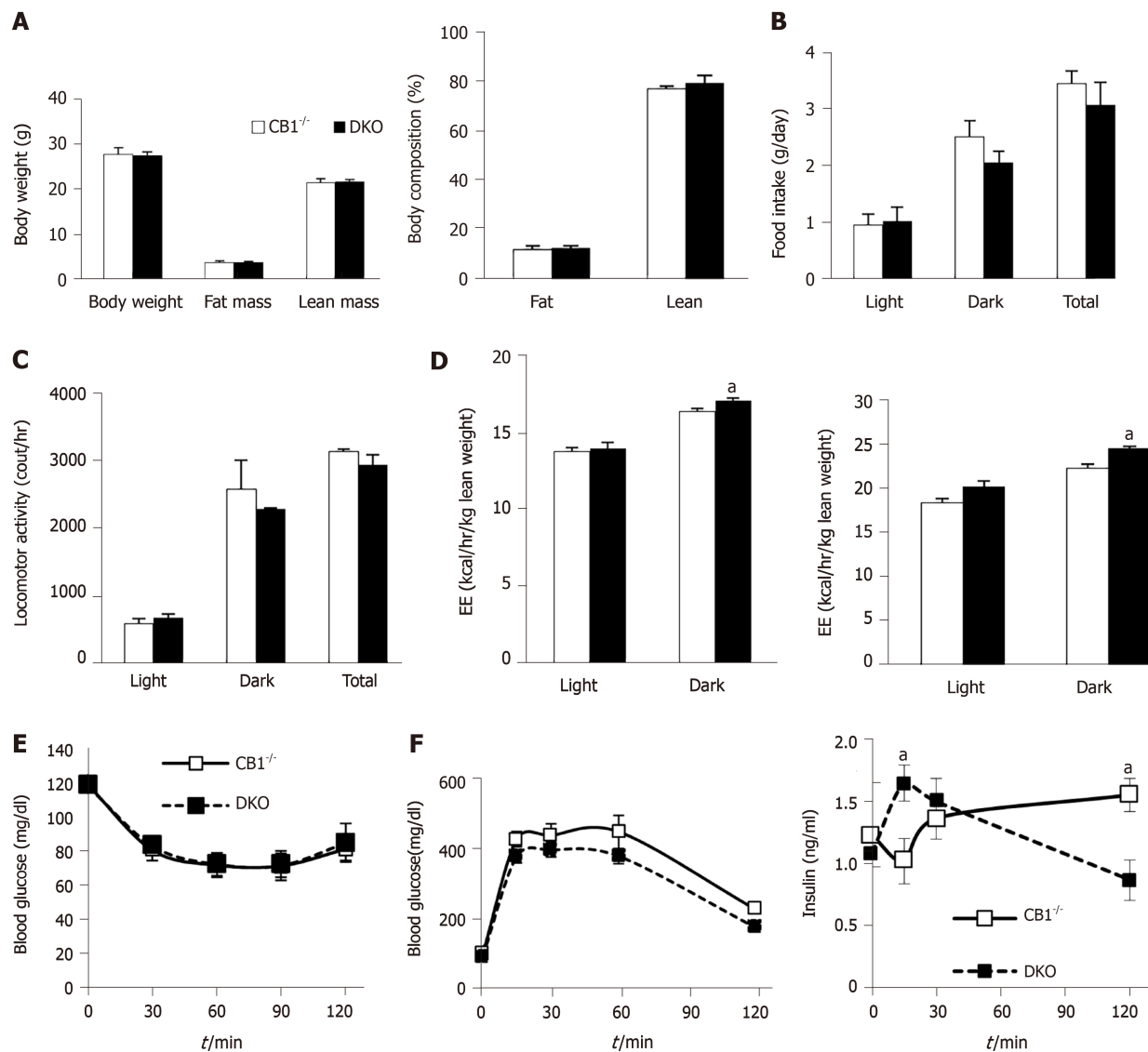
Figure 2 Cannabinoid type 1 receptor-null mice have similar adiposity and insulin sensitivity compared to double-knockout mice. Cannabinoid type 1 receptor-null (*CB1*^{-/-}) and double-knockout (DKO) male mice at 4 mo of age. A: Body weight and body composition; B: Daily food intake; C: Locomotor activity; D: Energy expenditure adjusted by body weight or lean mass; E: Insulin tolerance tests; F: Glucose tolerance tests at 5 mo of age. *n* = 5-7. [a]*P* < 0.05, *CB1*^{-/-} *vs* DKO.

regulation of *CB1* in insulin signaling, adipogenesis, and glucose uptake in BAT.

CB1 ablation promotes macrophage anti-inflammatory polarization

Macrophages have an important role in inflammation and insulin resistance[20]. To determine the underlying mechanisms of improved insulin sensitivity in *CB1*^{-/-} mice, we conducted flow cytometry analysis on peritoneal macrophages and adipose tissue macrophages. M1-like macrophages are pro-inflammatory and M2-like macrophages are anti-inflammatory[20]. Peritoneal M1-like macrophages, as well as the M1/M2 ratio as a readout of inflammation, were significantly decreased in *CB1*^{-/-}mice compared to WT mice; this suggests that *CB1* ablation reduces systemic inflammation (Figure 4A). Since insulin resistance is closely linked to adipose tissue mass and adipose macrophages (ATM), we next assessed epididymal white adipose tissue. As expected, both the weight and the ratio of epididymal fat/body weight was lower in *CB1*^{-/-} mice (Figure 4B). To assess the effect of *CB1* on ATM polarization, we isolated the stromal vascular fraction from epididymal adipose tissues. Our flow cytometry studies revealed that while M1 was slightly decreased, M2 was significantly increased in epididymal fat of *CB1*^{-/-} mice (Figure 4C). The M1/M2 ratio of ATM was decreased in epididymal fat of *CB1*^{-/-} mice (Figure 4C). Next, we studied the gene expression of macrophage markers of F4/80, CD11c, CD206, as well as proinflammatory cytokines of tumor necrosis factor-a (TNF-a), interelukin-1 (IL-1), and interelukin-6 (IL-6) in epididymal fat. The expression levels of F4/80, CD11c, CD206, TNF, IL-1 , and IL-6
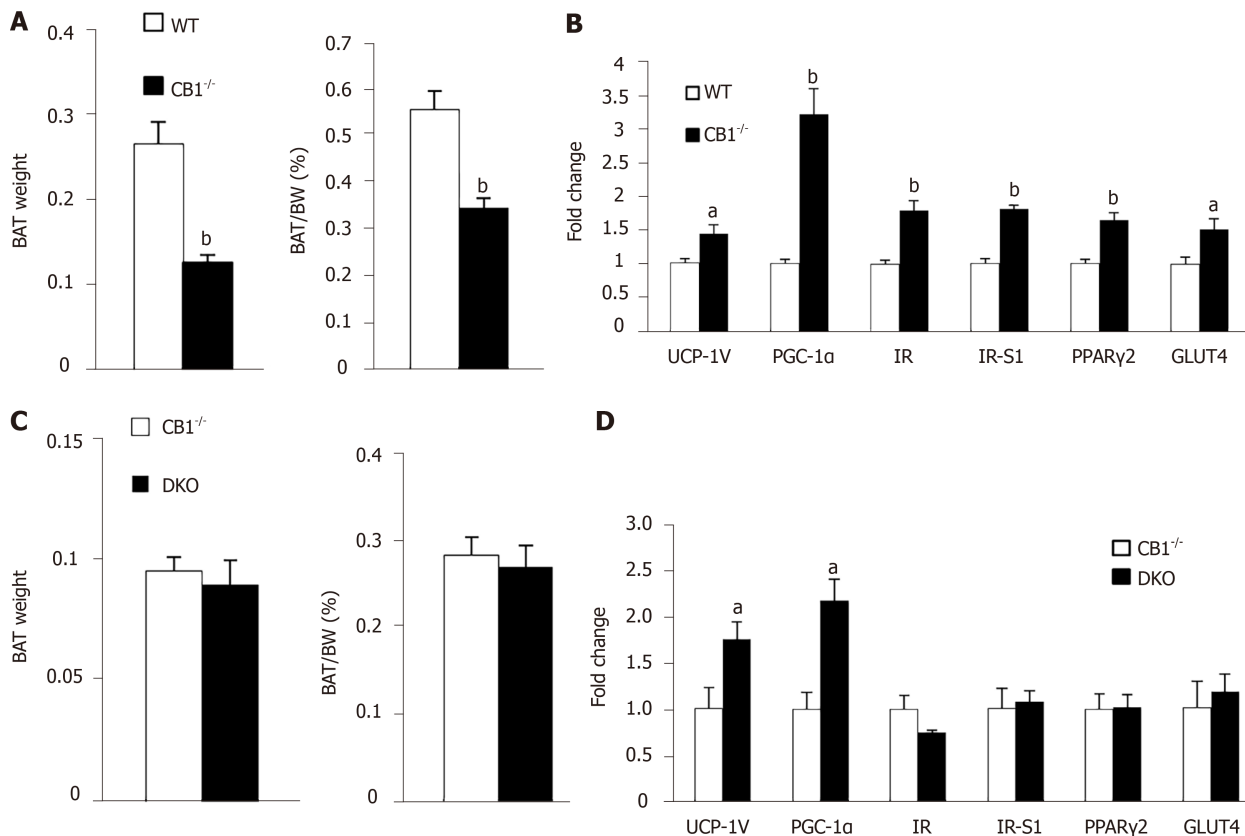
Figure 3 Double-knockout mice have similar expression of thermogenic genes compare to cannabinoid type 1 receptor-null mice. Wild-type (WT) and cannabinoid type 1 receptor-null (*CB1*^{-/-}) male mice at 8 mo of age. A: Brown adipose tissue (BAT) weight and percentage of BAT depot; B: Quantitative real-time RT-PCR analysis of gene expression in BAT. Cannabinoid type 1 receptor-null (*CB1*^{-/-}) and double-knockout (DKO) male mice at 8 mo of age. C: BAT weight and percentage of BAT depot; D: Quantitative real-time RT-PCR analysis of gene expression in BAT. *n* = 6-8. [a]*P* < 0.05, [b]*P* < 0.001, WT *vs CB1*^{-/-} or *CB1*^{-/-} *vs* DKO.

were significantly decreased in the epididymal fat of *CB1*^{-/-} mice compared to WT mice (Figure 4D), which is in agreement with the reduced inflammation revealed by flow cytometry analysis of ATM.

Adiponectin ablation abolishes the anti-inflammatory effect of CB1 deficiency

Adipose tissue releases adiponectin, which plays an important role in the regulation of energy metabolism and inflammation[24]. Intriguingly, our flow cytometry analysis showed increased pro-inflammatory peritoneal M1 macrophages and increased ratio of M1/M2 in DKO mice; this suggests that the adiponectin deletion abolishes the anti-inflammatory effect of *CB1* knockout (Figure 5A). Subsequently, we analyzed epididymal white adipose tissues of *CB1*^{-/-} and DKO mice. There was no difference in the percentage of fat depot: Body weight between *CB1*^{-/-} and DKO mice (Figure 5B). Our flow cytometry studies further revealed that the M1/M2 ratio of ATM was increased in epididymal fat of DKO mice compared to *CB1*^{-/-} mice (Figure 5C). To investigate the effect of adiponectin ablation of *CB1* on ATM-mediated inflammation, gene expressions of proinflammatory cytokines were evaluated in epididymal fat. The expression levels of F4/80, CD11c, CD206, TNF-a, IL-1, IL-6 and MCP-1 were significantly increased in epididymal fat of DKO mice as compared to *CB1*^{-/-} mice (Figure 5D), in line with increased inflammation observed by flow cytometry.

Collectively, the data indicate that the *CB1* deficiency-induced anti-inflammatory effect on macrophage polarization is adiponectin-dependent, suggesting that adiponectin is a key mediator for the effect of *CB1* on inflammation.

DISCUSSION

The *CB1* blockade has been shown to ameliorate metabolic abnormalities of obese animals and to promote weight loss and improved insulin sensitivity[25]. Adipokine adiponectin is an insulin-sensitizer, and it has many beneficial effects that phenocopy
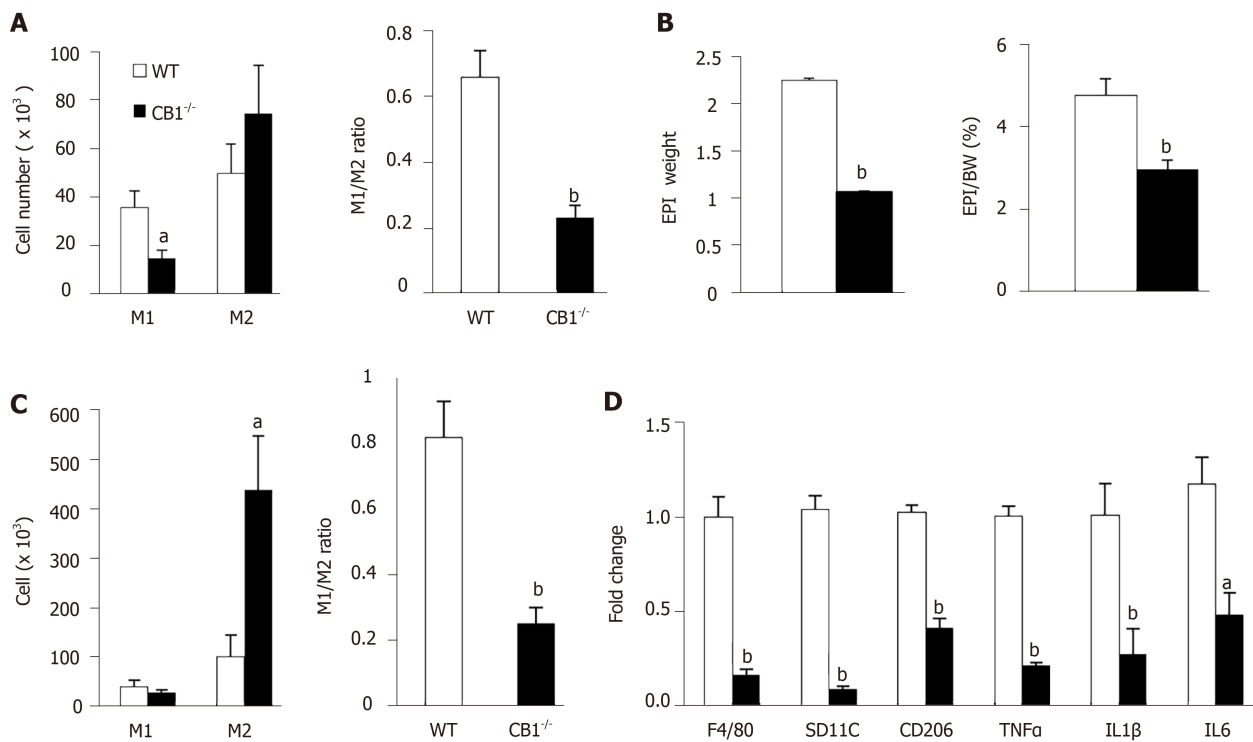
Figure 4 Cannabinoid type 1 receptor-null mice have reduced peritoneal and adipose tissue inflammation. Wild-type (WT) and cannabinoid type 1 receptor-null (*CB1*^{-/-}) male mice at 8 mo of age. A: Flow cytometry analysis of M1 and M2 in peritoneal macrophages; B: Percentage of epididymal fat depot; C: Flow cytometry analysis of M1 and M2 in epididymal fat; D: Pro-inflammatory cytokines expression in epididymal fat. *n* = 6-8. [a]*P* < 0.05, [b]*P*<0.001, WT *vs CB1*^{-/-} mice.

CB1 antagonists[12]. It has been shown that cannabinoids decrease adiponectin[26]. Moreover, *CB1* blocker rimonabant has been reported to increase the plasma adiponectin levels in obese and diabetic animal models[6,27,28]. Thus, adiponectin is thought to be a mediator of the effects of *CB1* antagonists such as rimonabant. However, the functional relationship between adiponectin and the endocannabinoid system is not fully defined. To determine whether *CB1* and adiponectin are functionally dependent on each other, we conducted a comparative study of the *CB1*^{-/-} and DKO mice to investigate whether the adiponectin deletion abolishes the healthy phenotype of *CB1*^{-/-} in metabolism and inflammation.

As expected in *CB1*^{-/-} mice, we observed decreased body weight**:**fat mass, increased thermogenic activation in BAT, and improved whole-body insulin sensitivity. Interestingly, DKO mice showed changes similar to *CB1*^{-/-} mice in the body weight**:**fat mass ratio, BAT thermogenic regulation, and insulin sensitivity. These results suggest that the beneficial metabolic effects of *CB1* blockage are not mediated by adiponectin. Our findings are mostly consistent with previous reports in literature, but with some differences which could be due to models of choice and/or diet variations. Watanabe *et al*[16] reported that rimonabant improved hepatic insulin resistance in both *ob/ob* and *adiponectin*^{-/-}*ob/ob* mice. Migrenne *et al*[29] reported that adiponectin is not required for body weight loss in diet-induced obese mice, but is required in rimonabant-induced improvement of insulin sensitivity. Our experiment was conducted with a genetic approach of loss-of-function with *CB1* knockout, not with *CB1* antagonist; under regular diet-feeding, not diet-induced obesity. It is possible that the impact of adiponectin on *CB1* metabolic regulation differs under different metabolic states. Indeed, Tam *et al*[30] reported a reversal of the HFD-induced hepatic steatosis and fibrosis by chronic administration of *CB1* blocker or adiponectin, but the reduction of adiposity and improved glycemic control are not affected by adiponectin, which is similar to our results.

The findings from our current study and others[4,8] support the idea of increased energy expenditure induced by *CB1* suppression, either by *CB1* blocker such as rimonabant or by *CB1* gene ablation. It is well known that BAT plays an important role in adaptive thermogenesis, and that thermogenic activation of BAT can directly affect metabolic rate through the function of mitochondrial protein UCP1. UCP1 is a key regulator of thermogenesis; it recruits free fatty acid into the mitochondrial matrix to dissipate as heat, depleting circulating lipids and increasing energy expenditure[31]. Previous studies demonstrated that rimonabant treatment increased the expression of
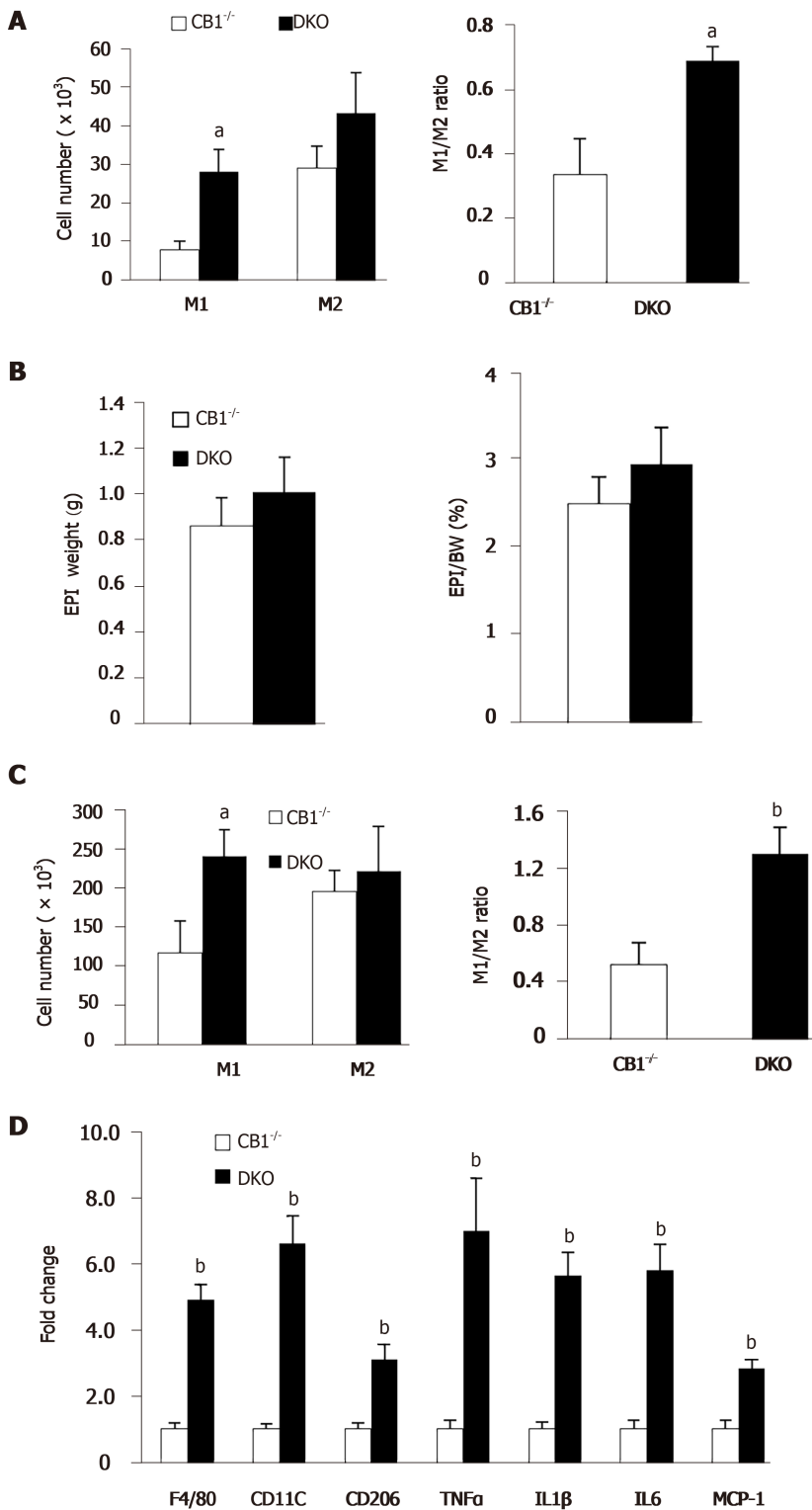
Figure 5 Double-knockout mice have increased peritoneal and adipose tissue inflammation compared to cannabinoid type 1 receptor-null mice. Cannabinoid type 1 receptor-null (*CB1⁻/⁻*) and double-knockout (DKO) male mice at 7 mo of age. A: Flow cytometry analysis of M1 and M2 macrophages of peritoneal macrophages; B: Epididymal weight and percentage of epididymal depot; C: Flow cytometry analysis of M1 and M2 in stromal vascular fraction of epididymal fat; D: Pro-inflammatory cytokines expression of epididymal fat. *n* = 6-7. ªP < 0.05, ᵇP < 0.001, *CB1⁻/⁻ vs* DKO mice.

UCP1 mRNA in BAT[32]. In metabolic profiling, DKO mice showed even higher energy expenditure than *CB1⁻/⁻* mice. Similarly, UCP-1 expression in BAT was higher in DKO mice than in *CB1⁻/⁻* mice. These results suggest that adiponectin deletion not diminishes the *CB1* deficiency-induced thermogenic activation in BAT. In the current study, we found that insulin signaling IR and IRS-1 gene expression in BAT was increased in *CB1⁻/⁻* mice, and the expression of these genes was no different between DKO and *CB1⁻/⁻* mice. Our thermogenic gene expression data in DKO showed that

adiponectin deletion further enhanced the thermogenic activation compared to *CB1⁻/⁻* mice, implying that the effect of *CB1* on thermogenesis is largely independent of adiponectin. The effect of adiponectin on thermogenesis is an area of ongoing debate currently. Qiao *et al*[33] reported that adiponectin suppresses thermogenic action in BAT to reduce energy expenditure. We reported that the core body temperature of adiponectin-null mice was not affected under normal housing temperature but reduced under cold temperature, supporting that adiponectin is required for maintaining body temperature in cold[24]. Different from our previous report, our current study was conducted under room temperature, so it is not surprising that the effect of adiponectin on thermogenic activation of *CB1⁻/⁻* mice is minimal.

Since metabolism and insulin sensitivity are closely linked to inflammation, we further studied the role of *CB1* deficiency in macrophages. Remarkably, both systemic (peritoneal macrophages) and tissue macrophages (ATM) showed an anti-inflammatory polarization shift, supporting reduced inflammation in *CB1⁻/⁻* mice. Especially, *CB1⁻/⁻* mice exhibited decreased pro-inflammatory M1 macrophages in peritoneal macrophages, less epididymal fat mass, and reduced M1**/**M2 ratio and pro-inflammatory cytokine expression in the epididymal fat as compared to WT mice. The results indicate that *CB1⁻/⁻* mice have reduced adiposity and adipose inflammation, which is consistent with improved systemic insulin sensitivity. Intriguingly, our study further revealed that DKO mice had an opposite profile of increased inflammation compared to *CB1⁻/⁻* mice, which suggested that adiponectin deletion reversed the anti-inflammatory effect of *CB1* deletion. The DKO mice exhibited an increase in pro-inflammatory M1 macrophages and M1**/**M2 ratio for both peritoneal macrophages and ATM, as well as elevated pro-inflammatory cytokine expression in epididymal fat compared to *CB1⁻/⁻* mice. The anti-inflammatory effect on *CB1⁻/⁻* mice was reversed in the DKO mice clearly demonstrates that adiponectin is required for the anti-inflammatory benefit of *CB1* antagonism, and the inflammation phenotype of *CB1* is adiponectin-dependent. These exciting results suggest that adiponectin counters the pro-inflammatory effect of cannabinoids, and the beneficial anti-inflammatory effect of *CB1* antagonists is dependent on adiponectin. Indeed, data from a mouse model of adipocyte-specific deletion of the *CB1* gene lends support to our conclusion[34]. Plasma adiponectin levels were significantly increased in the adipocyte-specific *CB1*-deleted mice, and adipocyte-specific deletion of *CB1* was shown to be sufficient to protect against diet-induced obesity and promote anti-inflammatory polarization towards alternatively-activated M2 macrophages.

CONCLUSION

In conclusion, our study demonstrates that *CB1* deletion activates thermogenesis and suppresses inflammation *via* adiponectin-independent and adiponectin-dependent pathways, respectively (Figure 6). Based on our findings, we conclude that there are differential pathways and mechanisms by which *CB1* utilizes to regulate metabolism and inflammation; that the effects on metabolism are adiponectin-independent and the effects on inflammation are adiponectin-dependent. *CB1* deletion increases plasma adiponectin[30,35], which promotes anti-inflammatory polarization of macrophages, thereby promoting the beneficial anti-inflammatory effect. Adiponectin is not required for *CB1*-mediated metabolism, but is required for *CB1*-mediated inflammation. A better understanding of the signaling crosstalk between *CB1* and adiponectin would facilitate further therapeutic development of *CB1* antagonists. Our study provides new insights to the comprehensive connection between *CB1* and adiponectin for regulation of energy homeostasis, insulin sensitivity and inflammation.
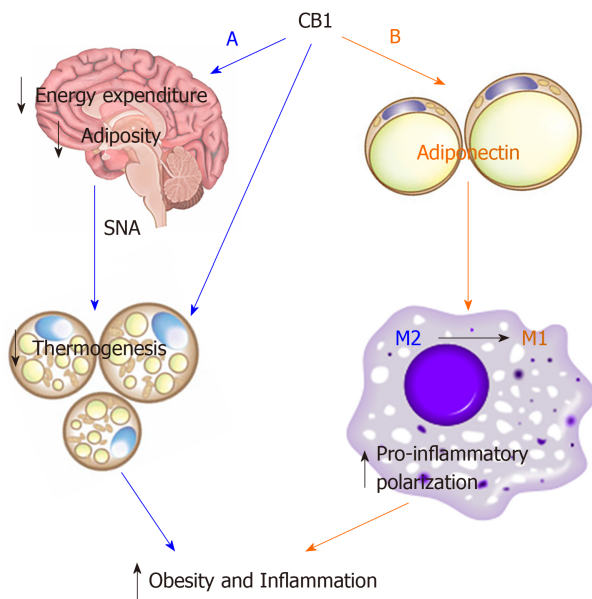
Figure 6 Schematic diagram of summary. Cannabinoid type 1 receptor (*CB1*) utilizes differential mechanisms in control of metabolism and inflammation. A: *CB1* decreases thermogenesis in BAT through sympathetic nerve activity to reduce energy expenditure and adiposity. So the effect of *CB1* on metabolism is adiponectin-independent; B: *CB1* suppresses adiponectin in adipose tissue, which diminishes the anti-inflammatory effect of adiponectin, thus promoting macrophage pro-inflammatory polarization. So the effect of *CB1* on inflammation is adiponectin-dependent. Thus, *CB1* utilizes differential mechanisms in control of metabolism and inflammation: its effect on metabolism is adiponectin-independent while effects on inflammation are adiponectin-dependent. Thus, adiponectin is not required for *CB1*-mediated metabolism, but is required for *CB1*-mediated inflammation.

ARTICLE HIGHLIGHTS

Research background
Antagonists of cannabinoid type 1 receptor (*CB1*) have been shown to promote body weight loss and improve insulin sensitivity.

Research motivation
Cannabinoids is implicated in regulation of adiponectin. However, the mediators of *CB1* actions are not fully defined, specifically in regard to adiponectin signaling *in vivo*.

Research objectives
To determine whether adiponectin is indeed required for the peripheral functions of *CB1*.

Research methods
We compared metabolic and inflammatory phenotypes of *CB1*-null (*CB1*$^{-/-}$) vs. *CB1*/Adiponectin double-knockout (DKO) mice. We investigated the insulin sensitivity using insulin tolerance test and glucose tolerance test, and inflammation using flow cytometry analysis of macrophages.

Research results
CB1$^{-/-}$ mice significantly reduced body weight and fat mass without change of total daily food intake and locomotor activity compared to wild-type (WT) mice. *CB1*$^{-/-}$ mice showed increased energy expenditure and improved insulin sensitivity compared to WT mice. DKO showed no difference in body weight, adiposity, or insulin sensitivity, only showed a modestly elevated thermogenesis in BAT compared to *CB1*$^{-/-}$ mice. *CB1*$^{-/-}$ mice showed reduced pro-inflammatory macrophage polarization in both peritoneal macrophages and adipose tissue macrophages compared to WT mice; in contrast, DKO mice exhibited elevated pro-inflammatory macrophage polarization in these macrophages compared to that of *CB1*$^{-/-}$ mice.

Research conclusions
Our findings reveal that *CB1* functions through both adiponectin-dependent and adiponectin-independent mechanisms: *CB1* regulates energy metabolism in an

adiponectin-independent manner, and inflammation in an adiponectin-dependent manner.

Research perspectives

Adiponectin is not required for *CB1*-mediated metabolism but is required for *CB1*-mediated inflammation. To fully understand the direct interactions and regulatory mechanisms between *CB1* and adiponectin, further dissemination in co-culture system to might be beneficial.

REFERENCES

1 **Blaha MJ**, Bansal S, Rouf R, Golden SH, Blumenthal RS, Defilippis AP. A practical "ABCDE" approach to the metabolic syndrome. *Mayo Clin Proc* 2008; **83**: 932-941 [PMID: 18674478 DOI: 10.4065/83.8.932]

2 **Tota-Maharaj R**, Defilippis AP, Blumenthal RS, Blaha MJ. A practical approach to the metabolic syndrome: review of current concepts and management. *Curr Opin Cardiol* 2010; **25**: 502-512 [PMID: 20644468 DOI: 10.1097/HCO.0b013e32833cd474]

3 **Williams CM**, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid (*CB1*) receptors. *Psychopharmacology (Berl)* 1999; **143**: 315-317 [PMID: 10353436 DOI: 10.1007/s002130050953]

4 **Di Marzo V**, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, Kunos G. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001; **410**: 822-825 [PMID: 11298451 DOI: 10.1038/35071088]

5 **Van Gaal LF**, Rissanen AM, Scheen AJ, Ziegler O, Rössner S; RIO-Europe Study Group. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 2005; **365**: 1389-1397 [PMID: 15836887 DOI: 10.1016/s0140-6736(05)66374-x]

6 **Després JP**, Golay A, Sjöström L; Rimonabant in Obesity-Lipids Study Group. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* 2005; **353**: 2121-2134 [PMID: 16291982 DOI: 10.1056/NEJMoa044537]

7 **Addy C**, Wright H, Van Laere K, Gantz I, Erondu N, Musser BJ, Lu K, Yuan J, Sanabria-Bohórquez SM, Stoch A, Stevens C, Fong TM, De Lepeleire I, Cilissen C, Cote J, Rosko K, Gendrano IN 3rd, Nguyen AM, Gumbiner B, Rothenberg P, de Hoon J, Bormans G, Depré M, Eng WS, Ravussin E, Klein S, Blundell J, Herman GA, Burns HD, Hargreaves RJ, Wagner J, Gottesdiener K, Amatruda JM, Heymsfield SB. The acyclic *CB1*R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. *Cell Metab* 2008; **7**: 68-78 [PMID: 18177726 DOI: 10.1016/j.cmet.2007.11.012]

8 **Cota D**, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thöne-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U. The endogenous cannabinoid system affects energy balance *via* central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 2003; **112**: 423-431 [PMID: 12897210 DOI: 10.1172/jci17725]

9 **Jbilo O**, Ravinet-Trillou C, Arnone M, Buisson I, Bribes E, Péleraux A, Pénarier G, Soubrié P, Le Fur G, Galiègue S, Casellas P. The *CB1* receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. *FASEB J* 2005; **19**: 1567-1569 [PMID: 16009704 DOI: 10.1096/fj.04-3177fje]

10 **Cardinal P**, Bellocchio L, Clark S, Cannich A, Klugmann M, Lutz B, Marsicano G, Cota D. Hypothalamic *CB1* cannabinoid receptors regulate energy balance in mice. *Endocrinology* 2012; **153**: 4136-4143 [PMID: 22778221 DOI: 10.1210/en.2012-1405]

11 **Boon MR**, Kooijman S, van Dam AD, Pelgrom LR, Berbée JF, Visseren CA, van Aggele RC, van den Hoek AM, Sips HC, Lombès M, Havekes LM, Tamsma JT, Guigas B, Meijer OC, Jukema JW, Rensen PC. Peripheral cannabinoid 1 receptor blockade activates brown adipose tissue and diminishes dyslipidemia and obesity. *FASEB J* 2014; **28**: 5361-5375 [PMID: 25154875 DOI: 10.1096/fj.13-247643]

12 **Lara-Castro C**, Fu Y, Chung BH, Garvey WT. Adiponectin and the metabolic syndrome: mechanisms mediating risk for metabolic and cardiovascular disease. *Curr Opin Lipidol* 2007; **18**: 263-270 [PMID: 17495599 DOI: 10.1097/MOL.0b013e32814a645f]

13 **Yamauchi T**, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941-946 [PMID: 11479627 DOI: 10.1038/90984]

14 **Combs TP**, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* 2001; **108**: 1875-1881 [PMID: 11748271 DOI: 10.1172/jci14120]

15 **Bensaid M**, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrié P. The cannabinoid *CB1* receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue

of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 2003; **63**: 908-914 [PMID: 12644592 DOI: 10.1124/mol.63.4.908]

16   **Watanabe T**, Kubota N, Ohsugi M, Kubota T, Takamoto I, Iwabu M, Awazawa M, Katsuyama H, Hasegawa C, Tokuyama K, Moroi M, Sugi K, Yamauchi T, Noda T, Nagai R, Terauchi Y, Tobe K, Ueki K, Kadowaki T. Rimonabant ameliorates insulin resistance *via* both adiponectin-dependent and adiponectin-independent pathways. *J Biol Chem* 2009; **284**: 1803-1812 [PMID: 19008231 DOI: 10.1074/jbc.M807120200]

17   **Obici S**, Wang J, Chowdury R, Feng Z, Siddhanta U, Morgan K, Rossetti L. Identification of a biochemical link between energy intake and energy expenditure. *J Clin Invest* 2002; **109**: 1599-1605 [PMID: 12070307 DOI: 10.1172/jci15258]

18   **Vasudevan AR**, Wu H, Xydakis AM, Jones PH, Smith EO, Sweeney JF, Corry DB, Ballantyne CM. Eotaxin and obesity. *J Clin Endocrinol Metab* 2006; **91**: 256-261 [PMID: 16263831 DOI: 10.1210/jc.2005-1280]

19   **Robker RL**, Collins RG, Beaudet AL, Mersmann HJ, Smith CW. Leukocyte migration in adipose tissue of mice null for ICAM-1 and Mac-1 adhesion receptors. *Obes Res* 2004; **12**: 936-940 [PMID: 15229332 DOI: 10.1038/oby.2004.114]

20   **Ma X**, Lin L, Yue J, Pradhan G, Qin G, Minze LJ, Wu H, Sheikh-Hamad D, Smith CW, Sun Y. Ghrelin receptor regulates HFCS-induced adipose inflammation and insulin resistance. *Nutr Diabetes* 2013; **3**: e99 [PMID: 24366371 DOI: 10.1038/nutd.2013.41]

21   **Lowell BB**, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature* 2000; **404**: 652-660 [PMID: 10766252 DOI: 10.1038/35007527]

22   **Inokuma K**, Ogura-Okamatsu Y, Toda C, Kimura K, Yamashita H, Saito M. Uncoupling protein 1 is necessary for norepinephrine-induced glucose utilization in brown adipose tissue. *Diabetes* 2005; **54**: 1385-1391 [PMID: 15855324 DOI: 10.2337/diabetes.54.5.1385]

23   **Kawashita NH**, Brito MN, Brito SR, Moura MA, Festuccia WT, Garofalo MA, Machado UF, Kettelhut IC, Migliorini RH. Glucose uptake, glucose transporter GLUT4, and glycolytic enzymes in brown adipose tissue from rats adapted to a high-protein diet. *Metabolism* 2002; **51**: 1501-1505 [PMID: 12404205 DOI: 10.1053/meta.2002.35582]

24   **Wei Q**, Lee JH, Wang H, Bongmba OYN, Wu CS, Pradhan G, Sun Z, Chew L, Bajaj M, Chan L, Chapkin RS, Chen MH, Sun Y. Adiponectin is required for maintaining normal body temperature in a cold environment. *BMC Physiol* 2017; **17**: 8 [PMID: 29058611 DOI: 10.1186/s12899-017-0034-7]

25   **Nogueiras R**, Veyrat-Durebex C, Suchanek PM, Klein M, Tschöp J, Caldwell C, Woods SC, Wittmann G, Watanabe M, Liposits Z, Fekete C, Reizes O, Rohner-Jeanrenaud F, Tschöp MH. Peripheral, but not central, *CB1* antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. *Diabetes* 2008; **57**: 2977-2991 [PMID: 18716045 DOI: 10.2337/db08-0161]

26   **Engeli S**. Peripheral metabolic effects of endocannabinoids and cannabinoid receptor blockade. *Obes Facts* 2008; **1**: 8-15 [PMID: 20054157 DOI: 10.1159/000114255]

27   **Poirier B**, Bidouard JP, Cadrouvele C, Marniquet X, Staels B, O'Connor SE, Janiak P, Herbert JM. The anti-obesity effect of rimonabant is associated with an improved serum lipid profile. *Diabetes Obes Metab* 2005; **7**: 65-72 [PMID: 15642077 DOI: 10.1111/j.1463-1326.2004.00374.x]

28   **Gary-Bobo M**, Elachouri G, Gallas JF, Janiak P, Marini P, Ravinet-Trillou C, Chabbert M, Cruccioli N, Pfersdorff C, Roque C, Arnone M, Croci T, Soubrié P, Oury-Donat F, Maffrand JP, Scatton B, Lacheretz F, Le Fur G, Herbert JM, Bensaid M. Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats. *Hepatology* 2007; **46**: 122-129 [PMID: 17526015 DOI: 10.1002/hep.21641]

29   **Migrenne S**, Lacombe A, Lefèvre AL, Pruniaux MP, Guillot E, Galzin AM, Magnan C. Adiponectin is required to mediate rimonabant-induced improvement of insulin sensitivity but not body weight loss in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2009; **296**: R929-R935 [PMID: 19211723 DOI: 10.1152/ajpregu.90824.2008]

30   **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* 2014; **306**: E457-E468 [PMID: 24381003 DOI: 10.1152/ajpendo.00489.2013]

31   **Cannon B**, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004; **84**: 277-359 [PMID: 14715917 DOI: 10.1152/physrev.00015.2003]

32   **Perwitz N**, Wenzel J, Wagner I, Büning J, Drenckhan M, Zarse K, Ristow M, Lilienthal W, Lehnert H, Klein J. Cannabinoid type 1 receptor blockade induces transdifferentiation towards a brown fat phenotype in white adipocytes. *Diabetes Obes Metab* 2010; **12**: 158-166 [PMID: 19895638 DOI: 10.1111/j.1463-1326.2009.01133.x]

33   **Qiao L**, Yoo Hs, Bosco C, Lee B, Feng GS, Schaack J, Chi NW, Shao J. Adiponectin reduces thermogenesis by inhibiting brown adipose tissue activation in mice. *Diabetologia* 2014; **57**: 1027-1036 [PMID: 24531262 DOI: 10.1007/s00125-014-3180-5]

34   **Ruiz de Azua I**, Mancini G, Srivastava RK, Rey AA, Cardinal P, Tedesco L, Zingaretti CM, Sassmann A, Quarta C, Schwitter C, Conrad A, Wettschureck N, Vemuri VK, Makriyannis A, Hartwig J, Mendez-Lago M, Bindila L, Monory K, Giordano A, Cinti S, Marsicano G, Offermanns S, Nisoli E, Pagotto U, Cota D, Lutz B. Adipocyte cannabinoid receptor *CB1* regulates energy homeostasis and alternatively activated macrophages. *J Clin Invest* 2017; **127**: 4148-4162 [PMID: 29035280 DOI: 10.1172/jci83626]

35   **Perwitz N**, Fasshauer M, Klein J. Cannabinoid receptor signaling directly inhibits thermogenesis and

alters expression of adiponectin and visfatin. *Horm Metab Res* 2006; **38**: 356-358 [PMID: 16718635 DOI: 10.1055/s-2006-925401]