Basic Study

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

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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.

Supported by the NIH, No. DK118334 and No. AG064869, and the BrightFocus, No. A2019630S (to Sun Y).

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

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.
RESPECTS

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

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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
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 CB1-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 C57/6] 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 °F; 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 O2 consumed. Respiratory quotient (RQ) ratio of VCO2/VO2 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 × 10⁶) 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 FACSscan 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 electrophoresis. 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 | CATTGATGCACTGACAGATGGA | CCGTACGGCATGAGGAAA     |
| IR     | CAAAAGCAACATCGAGATGAC  | ACCACGTGTGCAGTAAATCC   |
| IRS1   | GCCTGGAGTATTATGAAACGAA | GGGGATCGAGCGTTTGG      |
| PPARγ2 | GCCTATGAGCACTTCACAAGAA | TGCGAGTGGTCTTCCATCAC   |
| GLUT4  | GCCTTGGGAACACTCAACCA   | CACCTGGCACAACGAAATG    |
| F4/80  | GTTCGGCTATGCGCTTCCAGTC | GCACACTGTGCGGAACTC     |
| CD11c  | CTGGAGCTTCTTCTCGCTG    | GCCAGCTGTGCACTC        |
| CD206  | TGATTACGAGCAGTCCAAGGC  | GTTCACCGTAAGCCAAATT    |
| TNFα   | GAGAAGTCAACCTCCTCTCCTG | GAGACCTCCTGCCAGTTATG   |
| IL-1β  | TGTTCTGGAAGTGGACGCCACC | TCATCTGGAAGGCCTGAGTGC  |
| IL-6   | CCAGAGATCAAAGAAATGATG  | ACTCCAGAAGACCAGGAAT    |

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
transmembrane proton gradient and generates heat\(^2\)&\(^{21}\). UCP1 mRNA was increased in \(CB1^-\) mice as compared to WT controls (Figure 3B). Peroxisome proliferator-activated receptor-coactivator-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 receptors\(\gamma\)2 (PPAR\(\gamma\)2) is an important master adipogenic regulator \(^{11}\). Here we found that PPAR\(\gamma\)2 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 glucose 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 PPAR\(\gamma\)2 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
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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. *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
Double-knockout mice have similar expression of thermogenic genes compared 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. \(n = 6-8\). *\(P < 0.05\), **\(P < 0.001\), 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...
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 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.

ACKNOWLEDGMENTS

Metabolic analysis was performed in the Mouse Metabolic Research Unit at the USDA/ARS Children’s Nutrition Research Center, Baylor College of Medicine. The authors are very grateful to Michael R. Honig at Houston’s Community Public Radio Station KPFT for his excellent editorial assistance.
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 might be beneficial.

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