

# Genome-wide Expression Profiling Revealed Peripheral Effects of Cannabinoid Receptor 1 Inverse Agonists in Improving Insulin Sensitivity and Metabolic Parameters

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## ABSTRACT

Inhibition of cannabinoid receptor 1 (CB1) has shown efficacy in reducing body weight and improving metabolic parameters, with the effects correlating with target engagement in the brain. The peripheral effects of inhibiting the CB1 receptor has been appreciated through studies in diet-induced obese and liver-specific CB1 knockout mice. In this article, we systematically investigated gene expression changes in peripheral tissues of diet-induced obese mice treated with the CB1 inverse agonist AM251 [1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide]. CB1 receptor inhibition led to

down-regulation of genes within the de novo fatty acid and cholesterol synthetic pathways, including sterol regulatory element binding proteins 1 and 2 and their downstream targets in both liver and adipose tissue. In addition, genes involved in fatty acid  $\beta$ -oxidation were up-regulated with AM251 treatment, probably through the activation of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ). In adipose tissue, CB1 receptor inhibition led to the down-regulation of genes in the tumor necrosis factor  $\alpha$  signal transduction pathway and possibly to the activation of PPAR $\gamma$ , both of which would result in improved insulin sensitivity.

## Introduction

Obesity has become a global epidemic health problem, and it is estimated that in the United States one-third of the population is overweight or obese and 15% are morbidly obese (Bloom et al., 2008). However, current antiobesity pharmacotherapy demonstrates only moderate efficacy and is often associated with adverse effects (Van der Ploeg, 2000). Thus, there is a therapeutic need for safe and effective compounds for treating obesity.

Inhibition of the endocannabinoid receptor has shown promise in treating obesity and improving comorbidities (Bloom et al., 2008). Two cannabinoid receptor 1 (CB1) an-

tagonists/inverse agonists, rimonabant and taranabant, have gone through phase 3 clinical trials and proved to be efficacious in body weight reduction and improvement of metabolic parameters (Després et al., 2005; Van Gaal et al., 2005; Pi-Sunyer et al., 2006; Scheen et al., 2006; Addy et al., 2008). CB1 receptors are expressed predominantly in the central nervous system (CNS), which is believed to be the primary target tissue of rimonabant and taranabant. However, CNS engagement also is associated with psychiatric adverse effects, which limits the utility of CB1 antagonists/inverse agonists in treating obesity (Jones, 2008).

The CB1 receptor is also expressed at low levels in peripheral tissues such as liver and adipose (Bensaid et al., 2003; Osei-Hyiaman et al., 2005; Kunos et al., 2009). Several studies have investigated the hypothesis that the effects of CB1 receptor blockade on food intake and body weight may not be limited to a central mode of action. Antagonizing CB1 receptor suppresses the expression of the lipogenic transcription

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**ABBREVIATIONS:** CB1, cannabinoid receptor 1; SREBP, sterol regulatory element binding protein; ACC1, acetyl CoA carboxylase-1; FASN, fatty acid synthase; KO, knockout; WT, wild type; eWAT, epididymal white adipose tissue; DIO, diet-induced obese; RC, regular chow; HFD, high-fat diet; ANOVA, analysis of variance; FDR, false discovery rate; PPAR, peroxisome proliferator-activated receptor; CPT, carnitine palmitoyltransferase; TNF, tumor necrosis factor; TNFR, TNF receptor; TRAF, TNFR-associated factor; RIPK, receptor-interacting S/T kinase; CNS, central nervous system; MLYCD, malonyl CoA decarboxylase; AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide; SR141716, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide.

factor SREBP-1c and its target genes, acetyl CoA carboxylase-1 (ACC1) and fatty acid synthase (FASN), and de novo fatty acid synthesis both in vivo and in primary hepatocytes (Osei-Hyiaman et al., 2005). Hepatic de novo lipogenesis was decreased in liver-specific CB1 KO mice [LCB1(−/−)], as in the global-KO mouse (Osei-Hyiaman et al., 2008), underscoring the role of liver CB1 receptor in mediating the effect. LCB1(−/−) mice also showed more reduced liver steatosis, hyperglycemia, dislipidemia, and insulin and leptin resistance than wild-type (WT) controls, suggesting hepatic CB1 receptor signaling cascade contributes to the regulation of these metabolic pathways (Osei-Hyiaman et al., 2008).

To systematically investigate physiological pathways modulated by CB1 receptor inhibition in peripheral tissues, we performed gene expression profiling of liver and epididymal white adipose tissue (eWAT) isolated from lean and diet-induced obese (DIO) mice treated with a CB1 receptor inverse agonist. Our findings confirmed earlier reports of suppression of SREBP-1-responsive genes by CB1 inhibition and revealed regulation of additional pathways that should lead to improved metabolic parameters.

## Materials and Methods

**In Vivo Treatment for Transcriptional Profiling.** All animal experiments and euthanasia protocols were conducted in strict accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*. Animal experiment protocols were reviewed and approved by the Institutional Care and Use Committee of Merck Research Laboratories. The laboratory animal facilities of Merck Research Laboratories are certified by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Animals were housed in temperature-, humidity-, and light-controlled rooms (21–23°C, 47–65%, and 12/12-h light/dark cycle, respectively).

CB1(−/−) mice were obtained from A. Zimmer (University of Bonn, Bonn, Germany) (Zimmer et al., 1999) and back-crossed onto C57BL/6J genetic background for 10 generations by A. Zimmer before homozygous CB1(−/−) mice were rederived at Taconic Farms (Germantown, NY) onto the C57BL/6N genetic background. Male CB1(−/−) mice and control littermates ( $n = 5–7$  in each group) at 2 months of age were fed regular chow (RC) (Teklad 7012; 13% kcal from fat, 3.41 kcal/g; Harlan, Indianapolis, IN) or high-fat diet (HFD) (S3282; 59.4% kcal from fat; 24.5% kcal from carbohydrate; 16.2% kcal from protein; 5.29 kcal/g; Bio-Serv, Frenchtown, NJ) for 14 weeks and were individually caged 1 week before drug treatment. Vehicle (0.5% methylcellulose) or AM251 [1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide] (10 mg/kg; Sigma-Aldrich, St Louis, MO) were dosed by oral gavage at 5:00 PM daily for 2 days. Body weight was measured at 5:00 PM on days 1 and 2 and at 10:00 AM on day 3 before tissue collection. Food was measured at 5:00 PM on day 1 and 10:00 AM on day 3 before tissue collection. Food intake is calculated as the difference in food weight at the start of the study minus the food weight at the end of the study. Mice were euthanized by CO<sub>2</sub> asphyxiation at 10:00 AM after the second dose.

**RNA Extraction and Hybridization.** Total RNA was isolated from frozen tissues after homogenizing in TRIzol reagent (Invitrogen, Carlsbad, CA) and processed with RNeasy kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Samples were amplified and labeled by using a custom automated version of the RT/IVT protocol, and reagents were provided by Affymetrix (Santa Clara, CA). Hybridization, labeling, and scanning were completed following the manufacturer's recommendations (Affymetrix). Sample amplification, labeling, and microarray processing were performed at the Rosetta Inpharmatics Gene Expression Laboratory in

Seattle, WA. The raw gene expression data have been deposited in the Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo>; accession no. GSE21069).

**Statistical Analysis.** One-way ANOVA and false discovery rate (FDR) analyses were performed with MATLAB (The Mathworks, Inc., Natick, MA). Genes had to pass a prefilter of Affymetrix MAS5 present call ( $P < 0.05$ ) in >50% of the samples to qualify for further analysis. Ratios between a replicate and the virtual pool of control samples were calculated for each comparison. Differentially expressed genes (signature genes) were selected by ANOVA ( $P < 0.01$  or 0.05).

FDR was estimated by using Monte Carlo sample permutations. For each statistical test, a  $P$  value threshold ( $P < 0.01$ ) was used to select significant genes. Each sample was randomly permuted, changing the treatment group to which the sample belongs. This process was repeated 100 times. The FDR was calculated as the ratio of the average number of significant genes identified in the randomized data to the number of significant sequences in the original data.

**Pathway Analysis.** Modulation of a certain pathway can be significant if most genes in the pathway are regulated in the same direction, even though the expression change of an individual gene is small. To capture those small changes, we applied a relaxed cutoff to select signature genes ( $P < 0.05$  and absolute fold change >1.1) for pathway analysis. Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, CA) was used to analyze the enrichment of canonical pathways in the signature genes. A public microarray data set was queried with the NextBio System (NextBio, Cupertino, CA).

## Results

**AM251 Induced the Most Robust Gene Expression Changes in DIO Mice.** Previous studies demonstrated that the appetite- and weight-reducing effects of CB1 receptor inverse agonists are more profound in obese, hyperphagic animals than in lean controls (Hildebrandt et al., 2003; Vickers et al., 2003). Thus, it is possible that gene expression changes induced by CB1 inverse agonists depend on diet. We designed experiments that will not only allow the evaluation of diet effect, but also possible off-target activity of CB1 receptor inverse agonists (Table 1). Two-month-old male mice were fed RC or a HFD for 14 weeks and treated with either vehicle or AM251, a specific CB1 receptor inverse agonist (Gatley et al., 1996). CB1(−/−) mice were included to evaluate CB1 receptor-mediated effects versus non-mechanism-based activity of the compound. Two-day treatment with AM251 was sufficient to induce significant reduction in both food intake and body weight in WT but not CB1(−/−) mice. The effect was much stronger in mice fed a HFD. In DIO mice, a decrease of 74% food intake (Fig. 1A) and 8% body weight (Fig. 1C) was observed in DIO mice, whereas in lean mice a modest 17% reduction in food intake (Fig. 1B) and 3% decrease of BW (Fig. 1D) were detected. This was

TABLE 1

Design of gene expression profiling experiments

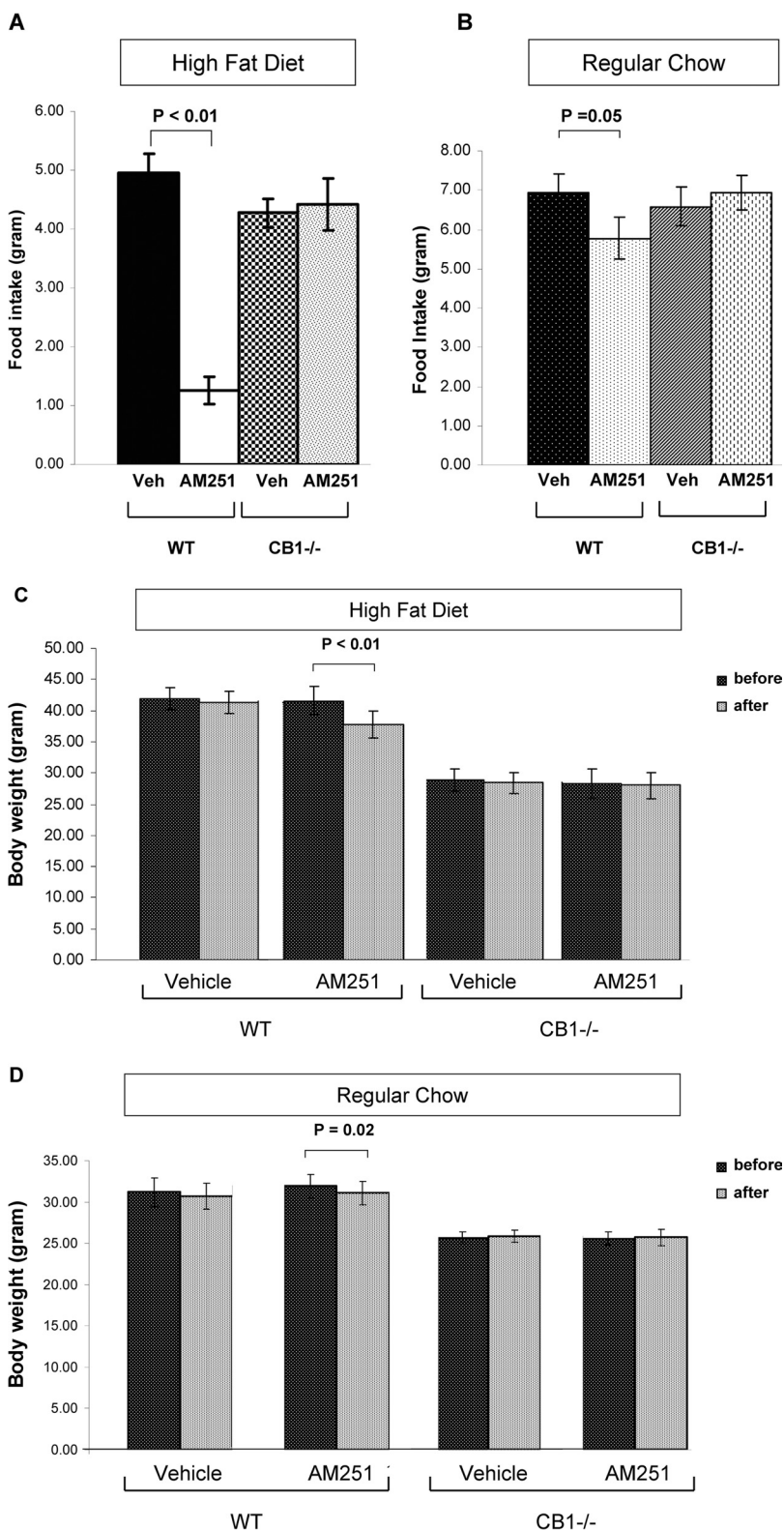
Male CB1(−/−) or WT mice were fed RC or a HFD for 14 weeks and treated with vehicle or AM251 for 2 days. Each treatment group has five to seven animals.

Diet	Genotype	Treatment	Number of Animals
HFD	WT	Vehicle	7
HFD	WT	AM251	7
HFD	CB1(−/−)	Vehicle	5
HFD	CB1(−/−)	AM251	6
RC	WT	Vehicle	7
RC	WT	AM251	7
RC	CB1(−/−)	Vehicle	5
RC	CB1(−/−)	AM251	6

consistent with earlier observations that the body weight reduction effect of CB1 inverse agonist was more robust in mice fed a HFD compared with those fed regular chow (Jbilo et al., 2005).

One-way ANOVA was performed between vehicle and AM251 treatment to define gene expression changes (signature genes) induced by AM251 in WT or CB1(-/-) mice fed

RC or a HFD (Table 2). In both liver and eWAT, AM251-induced gene expression changes were more robust in WT mice fed a HFD compared with those fed regular chow (Table 2). In liver with  $P$  value  $<0.01$  and absolute fold change  $>1.2$ , the AM251 signature size in WT DIO mice was more than 757 genes with 10% FDR, whereas in CB1(-/-) animals fed the same diet the signature size was only 101 with a much



**Fig. 1.** Food intake and body weight changes of mice treated with AM251 and vehicle (Veh) for 41 h. Student's  $t$  test was applied to evaluate significance. A and C, treatment with AM251 reduced food intake (A) and body weight (C) in WT DIO mice, but not in CB1(-/-) mice fed a HFD. B and D, decrease of food intake (B) and body weight (D) was also detected in lean WT mice, although the extent of reduction was less than that observed in DIO mice.



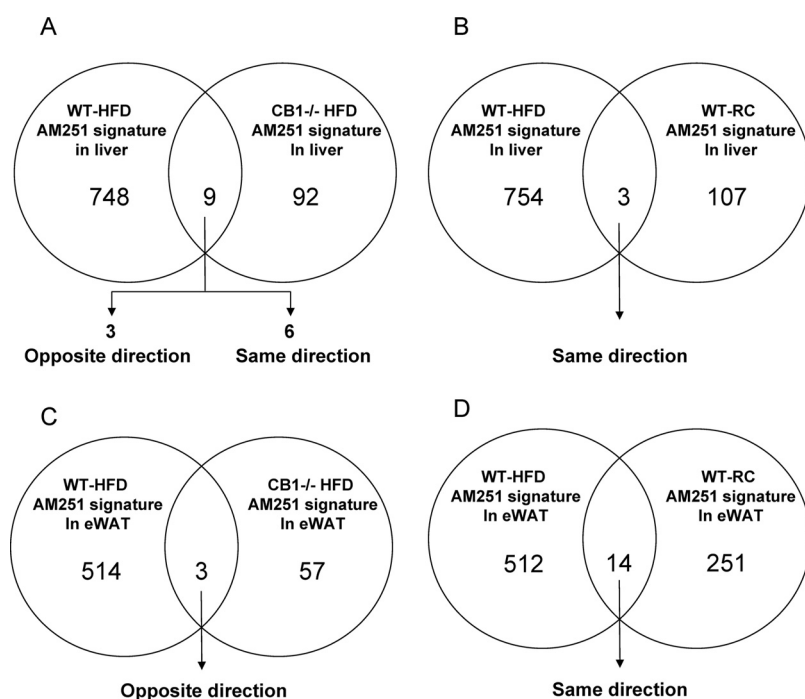
higher FDR (64%). There were only six commonly regulated genes between the WT and KO AM251 signatures under a HFD (Fig. 2A). Among those six genes, three belong to the cytochrome P450 family (*Cyp2b10*, *Cyp2c29*, and *Por*) and are perhaps involved in compound metabolism. The other three genes were heat shock protein (*Hspb1*), aminoacylase (*Aspa*), and a gene of unknown function (*4931406C07Rik*). These observations suggest minimal off-target effect of AM251. In lean mice, the effect of AM251 was much more modest (110 genes with 64% FDR) than in DIO mice under the same threshold (ANOVA  $P$  value  $<0.01$  and absolute fold change  $>1.2$ ), underlining the diet effect to transcriptional regulation of AM251. There were only three commonly regulated genes between the AM251 signatures in lean and DIO animals (Fig. 2B). The small overlap is not too surprising given the weak response and high false positive rate of AM251 signature in lean mice. Similar observations were made in eWAT, where no commonly regulated genes by AM251 between WT and CB1(−/−) mice were observed (both fed a HFD; Fig. 2C), and only 14 overlapping genes were found after AM251 treatment in either lean or DIO mice (Fig. 2D).

The experimental design also allowed for comparisons of

TABLE 2

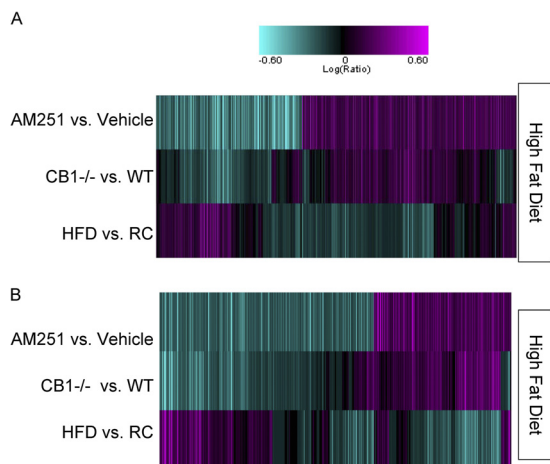
Gene expression changes induced by AM251 in each treatment group. One-way ANOVA was performed between vehicle- and AM251-treated animals in each comparison. Differentially expressed genes were selected with ANOVA  $P < 0.01$  and absolute fold change  $>1.2$ . FDR was estimated with Monte Carlo permutation.

Tissue	Comparison	Signature Size	FDR
			%
Liver	WT HFD: vehicle vs. AM251	757	10
Liver	CB1(−/−) HFD: vehicle vs. AM251	101	64
Liver	WT RC: vehicle vs. AM251	110	64
Liver	CB1(−/−) RC vehicle vs. AM251	133	66
eWAT	WT HFD: vehicle vs. AM251	526	20
eWAT	CB1(−/−) HFD: vehicle vs. AM251	69	100
eWAT	WT RC: vehicle vs. AM251	265	42
eWAT	CB1(−/−) RC: vehicle vs. AM251	141	60



**Fig. 2.** Venn diagrams of AM251 signatures (ANOVA  $P$  value  $< 0.01$  and absolute fold change  $>1.2$ ) between WT and CB1(−/−) mice and DIO and lean mice. A, in liver and under a HFD, there are only six overlapping AM251 signature genes between WT and CB1(−/−) mice, three of which belong to the cytochrome P450 family and are perhaps involved in AM251 metabolism. B, small overlap between AM251 signatures in WT DIO and lean mice in liver is observed. C, in eWAT under a HFD, no commonly regulated genes were found between WT and CB1(−/−) mice. D, there are 14 overlapping AM251 signature genes between WT DIO and lean mice in eWAT.

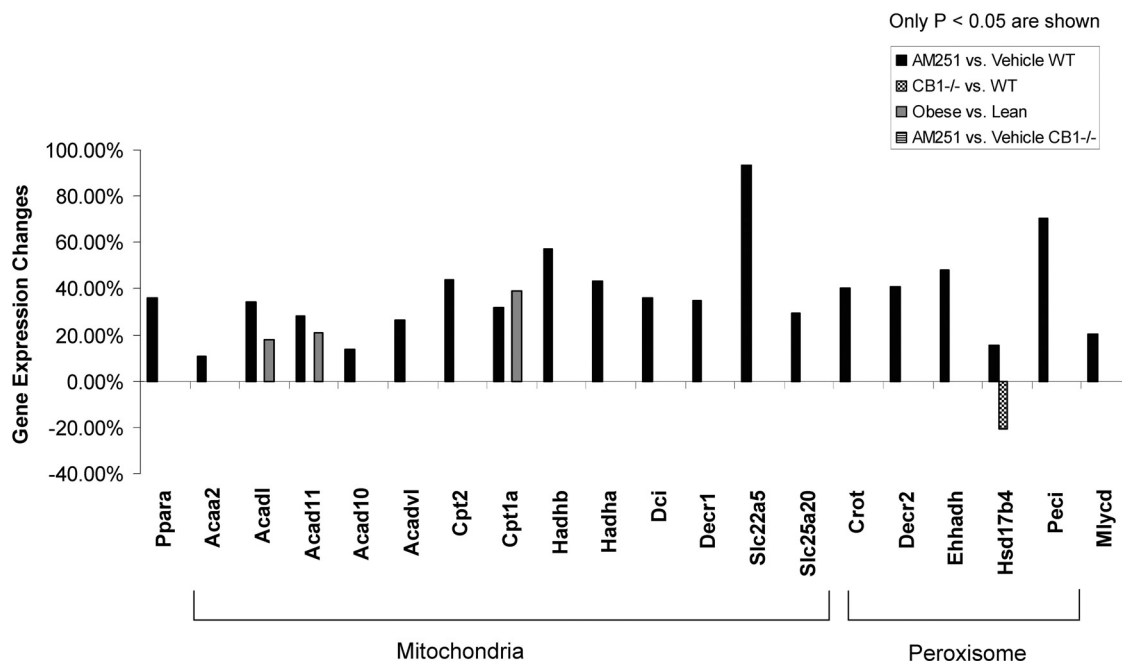
striped bars). None of these genes were significantly regulated in the livers of WT lean mice treated with AM251 (data not shown). Malonyl CoA decarboxylase (MLYCD), a target of PPAR $\alpha$  (Lee et al., 2004), was also up-regulated by AM251. An increase in MLYCD should decrease malonyl CoA levels, an inhibitor of CPT1a, and thus increase fatty acid  $\beta$ -oxidation (Ruderman et al., 1999). Osei-Hyiaman et al. (2008) demonstrated that CB1 receptor inverse agonist treatment increased liver CPT1a mRNA, protein level, and its enzymatic activity, also supporting the up-regulation of fatty acid  $\beta$ -oxidation by inhibition of the CB1 receptor.



**Fig. 3.** Many of the gene expression changes induced by 2-day treatment of AM251 in DIO mice are shared in CB1(-/-) mice (compared with WT) and are regulated in opposite directions by HFD feeding (compared with RC). Ratio experiments were generated with vehicle (top), WT (middle), or mice fed with RC (bottom) as the baselines. Shown in the clustergrams are log(ratio) values at a range of  $\pm 0.6$  (or  $\pm 4$ -fold change), with magenta and cyan signifying up- and down-regulation, respectively. A, liver. A total of 757 AM251 signature genes are shown (ANOVA  $P < 0.01$  and absolute fold change  $> 1.2$ ). B, eWAT. A total of 526 AM251 signature genes are shown (ANOVA  $P < 0.01$  and absolute fold change  $> 1.2$ ).

PPAR $\alpha$  regulates fatty acid  $\beta$ -oxidation in both mitochondria and peroxisome (Lefebvre et al., 2006; Reddy and Rao, 2006; Rakhshandehroo et al., 2007). The peroxisomal  $\beta$ -oxidation is more versatile than the mitochondrial pathway and is capable of metabolizing a wide variety of fatty acid analogs and very long chain fatty acid. Examination of the key enzymes catalyzing peroxisomal  $\beta$ -oxidation also reveals a trend of increased expression by AM251 treatment. The bifunctional enzyme composed of both enoyl-CoA hydratase and 3-hydroxylacyl CoA dehydrogenase (*Ehhadh*), carnitine *O*-octanoyltransferase (*Crot*), peroxisomal D3,D2-enoyl-CoA isomerase (*Peci*), peroxisomal 2,4-dienoyl CoA reductase 2 (*Decr2*), and hydroxysteroid (17- $\beta$ ) dehydrogenase 4 (*Hsd17b4*) were also increased by AM251 treatment in the livers of WT DIO mice (Fig. 4, black bars), but not CB1(-/-) mice fed a HFD (Fig. 4, striped bars).

**De Novo Fatty Acid and Cholesterol Synthesis Pathways Are Suppressed by AM251.** Osei-Hyiaman et al. (2005) demonstrated earlier that the CB1 receptor antagonist decreased expression of the lipogenic transcription factor SREBP-1c (an isoform of SREBP-1) and its target genes ACC1 and FASN. The suppression is accompanied by a reduction in de novo fatty acid synthesis in liver, both in vivo and in vitro (Osei-Hyiaman et al., 2005). Our microarray data not only confirmed their observation, but also showed the down-regulation of additional SREBP-1 target genes crucial for lipogenesis. These include stearyl CoA desaturase 1 (*Scd1*), long-chain fatty-acid elongase 5 and 6 (*Elovl5* and *Elovl6*), ATP citrate lyase (*Acl*), fatty acid desaturase 1 and 2 (*Fads1* and *Fads2*), and glycerol-3-phosphate acyltransferase (*Gpam*) (Fig. 5A, black bars). In CB1(-/-) mice, expression of SREBP-1 and some of its target genes were also decreased compared with WT mice (Fig. 5A, dotted bars), although the extent of regulation is not as profound as in AM251 treatment. This is probably attributed to the difference between acute inhibition of CB1 receptor versus the



**Fig. 4.** In liver, PPAR $\alpha$  and its target genes regulating mitochondrial and peroxisomal fatty acid  $\beta$ -oxidation are increased by AM251 treatment in WT DIO mice (black bars), but not in CB1(-/-) mice fed a HFD (striped bars). The y-axis represents percentage of gene expression changes as measured by microarray. Only genes with significant expression changes (ANOVA  $P < 0.05$ ) are shown.

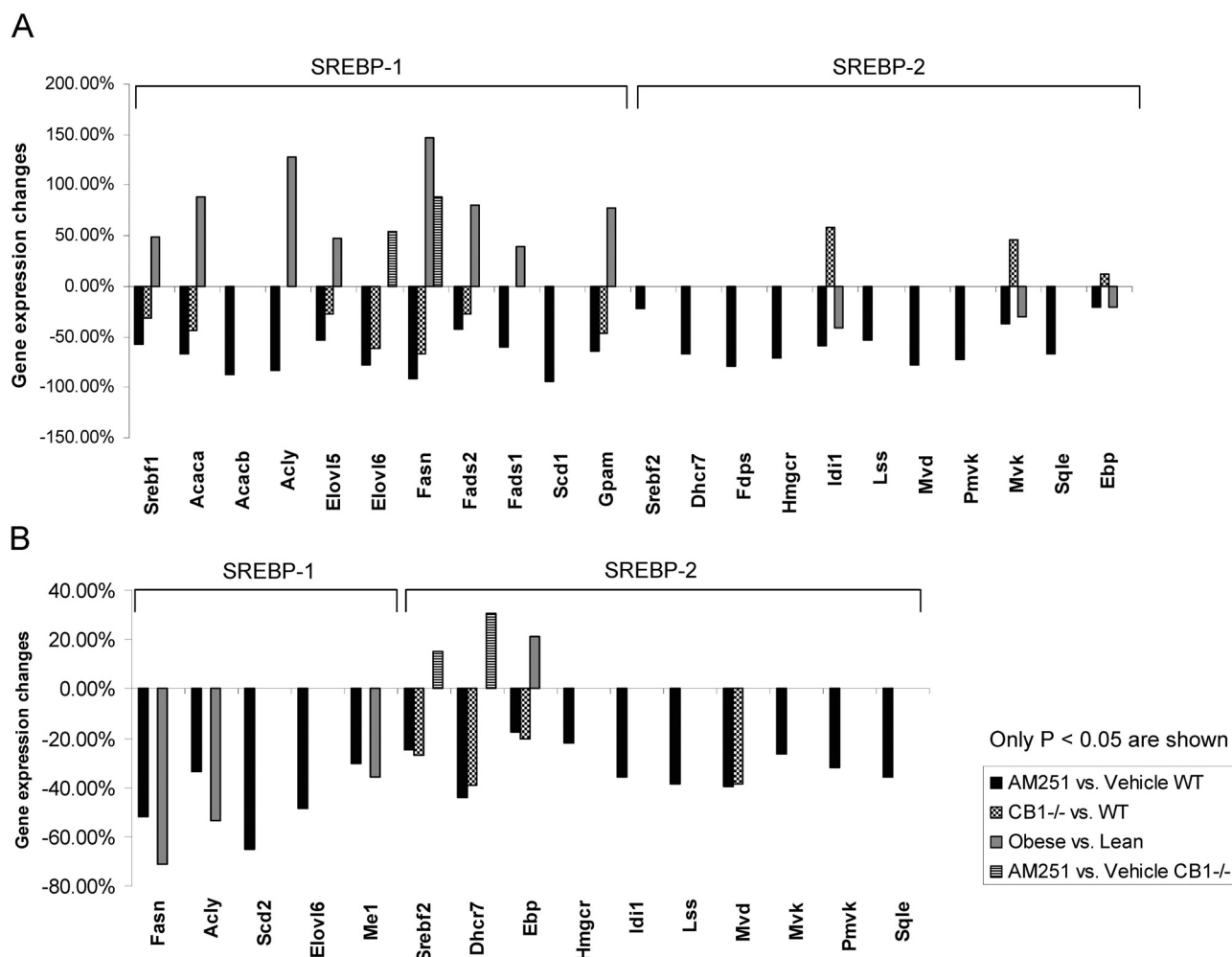
more chronic effect caused by embryonic inactivation of the gene. The latter could induce compensatory effect for the loss of CB1 receptor activity. In mice fed a high-fat diet, the SREBP-1 responsive genes were up-regulated compared with those on regular chow, which probably is reflective of steatosis observed in obese animals. The SREBP-1 gene encodes two members, SREBP-1a and SREBP-1c, through the use of alternative transcription start site, and SREBP-1c is a weaker transcription activator than SREBP-1a (Brown and Goldstein, 1997; Shimano et al., 1997). The SREBP-1 probe on our Affymetrix chip was designed from the 3' untranslated region, which is shared by SREBP-1a and SREBP-1c; thus, we were not able to discern the two isoforms.

The SREBP-1 isoforms are more selective in activating fatty acid biosynthetic genes, whereas SREBP-2 is more specific for controlling cholesterol biosynthesis (Shimano et al., 1999). In our experiments, SREBP-2 was also decreased by AM251 treatment, accompanied by down-regulation of cholesterol synthesis genes such as HMG CoA reductase (*Hmgcr*), farnesyl diphosphate synthase (*Fdps*), and squalene synthase (*Sqle*) (Fig. 5A, black bars). Again, this suppression was mediated by the CB1 receptor and was not observed in CB1(−/−) mice treated with AM251 (Fig. 5, striped bars).

It is noteworthy that the down-regulation of SREBP-2 and

its target genes was not limited to liver. In eWAT, SREBP-2 and genes involved in cholesterol synthesis were also decreased by AM251 (Fig. 5B, black bars). Although SREBP-1 was not significantly regulated in eWAT, some of its target genes [e.g., *Fasn*, ATP citrate lyase (*Acly*), long-chain fatty-acid elongase 6 (*Elovl6*), cytosolic malic enzyme (*Me1*), and stearyl CoA desaturase 2 (*Scd2*)] were decreased by AM251. It is intriguing that the SREBP pathways were similarly regulated by the CB1 receptor inverse agonist in both liver and eWAT, although the mechanism leading to this down-regulation of SREBPs remains to be characterized.

**TNF $\alpha$  Signaling Pathway Is Decreased by AM251 in Epididymal White Adipose Tissue.** Similar to the observation in liver, AM251 treatment reversed many gene expression changes caused by HFD treatment in eWAT (Fig. 3B, top and bottom). In addition, the AM251 signature in eWAT has significant overlap with the gene expression changes between WT and CB1(−/−) mice (Fig. 3B, top and middle), suggesting these transcriptional regulations are mediated by the CB1 receptor. Pathway analysis of the signature genes was performed to investigate physiological pathways perturbed by these expression changes. One of the top hits was the TNF $\alpha$  signaling pathway. Activation of this pathway in adipose tissue is associated with insulin resis-



**Fig. 5.** SREBP-1 and SREBP-2 target genes are suppressed by AM251 treatment in WT mice (black bars), but not in CB1(−/−) mice (striped bars). A, Liver. B, eWAT. Only genes with significant expression changes ( $P < 0.05$ ) are shown. In liver, SREBP-1 and target genes are also decreased in CB1(−/−) mice compared with WT controls (dotted bars), whereas they are up-regulated by a high-fat diet compared with regular chow (gray bars).

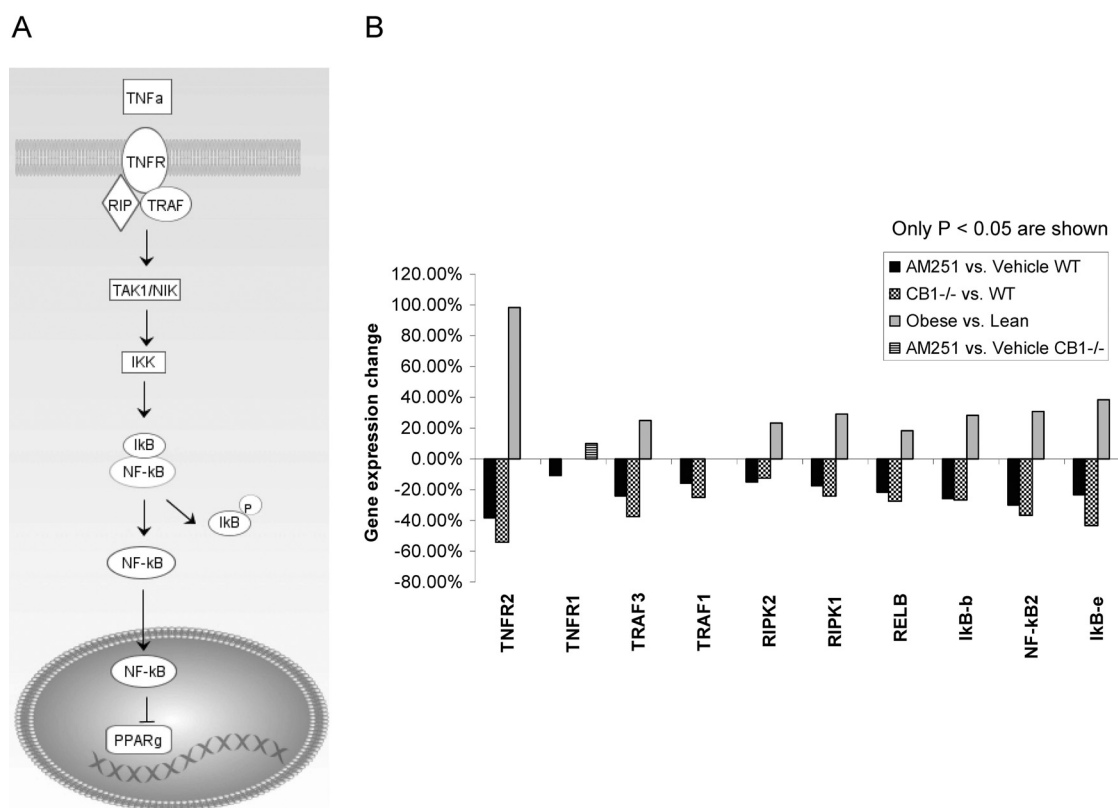


tance, and  $\text{TNF}\alpha$  has been shown to suppress  $\text{PPAR}\gamma$  activity, impair insulin signaling, and increase lipolysis in adipocytes (Guilherme et al., 2008). In DIO mice, genes mediating the  $\text{TNF}\alpha$  signaling pathway (Hayden and Ghosh, 2008) (Fig. 6A) were up-regulated compared with lean mice (Fig. 6B, gray bars): these include increases in gene expression of the  $\text{TNF}$  receptors ( $\text{TNFRs}$ ), receptor-interacting S/T kinases ( $\text{RIPKs}$ ),  $\text{TNF}$  receptor-associated factors ( $\text{TRAFs}$ ), the  $\text{NF-}\kappa\text{B}$  family of transcription factors ( $\text{NF-}\kappa\text{B2}$  and  $\text{RELB}$ ), and the  $\text{I}\kappa\text{B}$  family members ( $\text{I}\kappa\text{B-b}$  and  $\text{I}\kappa\text{B-e}$ ). Treatment with AM251 reversed these regulations and decreased the  $\text{TNF}\alpha$  signaling pathway (Fig. 6B, black bars). In  $\text{CB1}(-/-)$  mice, the  $\text{TNF}\alpha$  pathway was also decreased compared with WT mice (Fig. 6B, dotted bars), further supporting that the regulation was mediated through the  $\text{CB1}$  receptor.

**$\text{PPAR}\gamma$  Mediates Part of the AM251 Effect in Adipose Tissue.**  $\text{PPAR}\gamma$  is a key nuclear receptor regulating adipocyte differentiation and lipid storage, and its activation leads to an improvement in insulin sensitivity in diabetic patients and animal models (Berger et al., 2005).  $\text{TNF}\alpha$  decreases  $\text{PPAR}\gamma$  activity at multiple levels, including the transcription level (Zhang et al., 1996; Ruan et al., 2002a,b). Although we did not detect a significant change of  $\text{PPAR}\gamma$  expression level, a handful of  $\text{PPAR}\gamma$  target genes, such as  $\text{PEPCK}$ ,  $\text{CD36}$ ,  $\text{FATP-1}$ ,  $\text{UCP3}$ , and  $\text{PDK4}$  (Tontonoz et al., 1995; Martin et al., 1997; Sfeir et al., 1997; Kelly et al., 1998; Berger and Moller, 2002) all were up-regulated in eWAT by AM251 treatment of DIO mice. Muise et al. (2008) reported systematic identification of  $\text{PPAR}\gamma$  agonist-responsive genes in the

eWAT of *dbdb* mice via transcriptional profiling of two structurally distinct  $\text{PPAR}\gamma$  agonists. We compared the AM251 eWAT signature in DIO mice (a total of 1553 genes with ANOVA  $P < 0.05$  and absolute fold change  $>1.1$ ) to the  $\text{PPAR}\gamma$  agonist signature in the eWAT of *dbdb* (339 genes) as reported previously (Muise et al., 2008) and found a significant overlap (a total of 80 genes with a hypergeometric  $P < 4\text{E-}15$ ). A few of these genes are listed in Table 3. Consistent with speculation that AM251 suppressed  $\text{TNF}\alpha$  signaling and thus an increase of  $\text{PPAR}\gamma$  target genes, many genes activated by  $\text{PPAR}\gamma$  agonists were also increased by AM251 treatment in the eWAT of DIO mice. These include genes regulating lipid uptake ( $\text{CD36}$  and  $\text{FATP-1}$ ), glycerol synthesis/lipogenesis ( $\text{PEPCK1}$  and  $\text{PDK4}$ ), lipoprotein hydrolysis ( $\text{MGLL}$ ), and fatty acid  $\beta$ -oxidation and energy expenditure ( $\text{MLYCD}$ ,  $\text{ACAA1B}$ , and  $\text{UCP3}$ ). However, genes catalyzing de novo fatty acid synthesis were differentially regulated between AM251 and  $\text{PPAR}\gamma$  agonists.  $\text{FASN}$  and cytosolic malic enzyme ( $\text{ME1}$ ) were increased by  $\text{PPAR}\gamma$  agonist treatment, but decreased by AM251 treatment. The down-regulation of de novo fatty acid synthesis genes observed in AM251 treatment probably was caused by  $\text{SREBP}$  suppression and the different animal models used (DIO versus *dbdb*).

$\text{PPAR}\gamma$  also modulates the secretion of several adipokines that function as hormones and regulate insulin sensitivity and energy homeostasis. Leptin was reported to be negatively regulated by  $\text{PPAR}\gamma$ , and we also detected a decrease of leptin gene expression in AM251 treatment. Adiponectin is another hormone secreted by adipocytes and stimulates fatty acid oxidation in liver through AMP-activated protein kinase



**Fig. 6.** In eWAT, expression of genes in the  $\text{TNF}\alpha$  signaling pathway (A) is increased by a HFD relative to RC feeding (B, gray bars). Two-day treatment of DIO mice with AM251 decreased gene expression of this pathway (B, black bars). The  $\text{TNF}\alpha$  signaling pathway is also down-regulated in  $\text{CB1}(-/-)$  mice fed a HFD compared with WT DIO mice (B, dotted bars). Only significant gene expression changes (ANOVA  $P < 0.05$ ) are shown.

(Yamauchi et al., 2002), and it is also a downstream target of PPAR $\gamma$  (Nawrocki et al., 2006; Muise et al., 2008). It has been reported that AM251 treatment leads to an increase in adiponectin mRNA level in adipose tissue and adipocytes (Bensaid et al., 2003; Jbilo et al., 2005). We were unable to detect gene expression changes of adiponectin, probably because of the relative short treatment of AM251 in our studies (2 days). In the article published by Bensaid et al., a significant increase of adiponectin mRNA was detectable only after at least 4 days of treatment with the CB1 receptor inverse agonist.

## Discussion

Increasing experimental and clinical data are demonstrating peripheral effects of CB1 inverse agonists. To understand the molecular mechanisms underlying this we performed genome-wide gene expression analysis of lean and DIO mice treated with the CB1 inverse agonist AM251. We demonstrate that AM251 induced robust signatures in DIO mice but had much more modest effects in lean mice. This is consistent with earlier reports that the

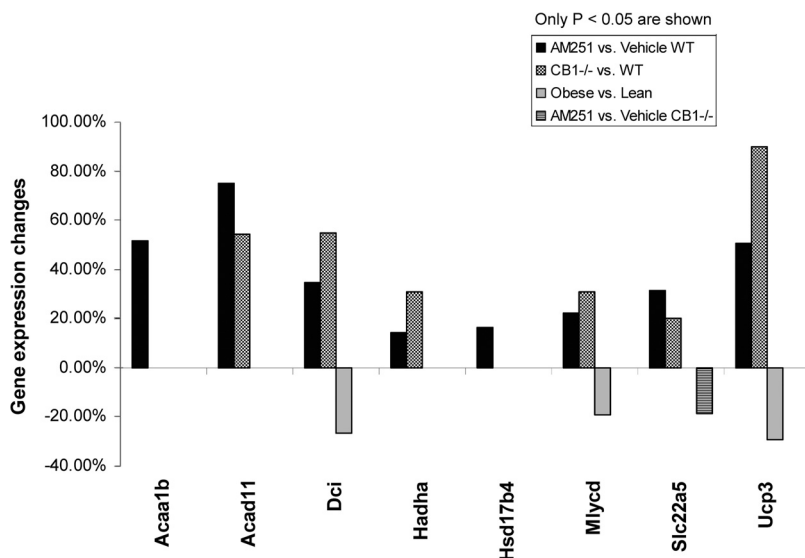
TABLE 3

Many PPAR $\gamma$  target genes are regulated similarly by AM251 and PPAR $\gamma$  agonists in eWAT

AM251 signature genes (ANOVA  $P < 0.05$  and absolute fold change  $> 1.1$ ) were compared with PPAR $\gamma$  target genes reported in literature (Tontonoz et al., 1995; Martin et al., 1997; Sfeir et al., 1997; Kelly et al., 1998; Berger and Moller, 2002; Muise et al., 2008). Genes regulated in opposite directions are in bold. Regulation of these genes in CB1(–/–) animals compared with WT controls are also included; many of the genes are regulated in the same trend between AM251 treatment and CB1 knockout.

Pathway	Gene Symbol	AM251	PPAR $\gamma$ Agonists	CB1(–/–)
Fatty acid transport	CD36	Up	Up	N.C.
Fatty acid transport	FATP-1	Up	Up	Up
Lipoprotein hydrolysis	MGLL	Up	Up	Up
Lipid synthesis	FASN	Down	Up	N.C.
Lipid synthesis	ME1	Down	Up	N.C.
Lipid synthesis	PEPCK	Up	Up	Up
Lipid synthesis	PDK4	Up	Up	N.C.
Adipokine	LEP	Down	Down	Down
Fatty acid $\beta$ -oxidation	MLYCD	Up	Up	Up
Fatty acid $\beta$ -oxidation	ACAA1B	Up	Up	N.C.
Energy expenditure	UCP3	Up	Up	Up

N.C., no change (genes that did not show significant expression changes).



**Fig. 7.** Genes catalyzing fatty acid  $\beta$ -oxidation and energy expenditure are increased by AM251 treatment in the eWAT of WT DIO mice (black bars), but not in CB1(–/–) mice fed a HFD (striped bars). Most of these genes are regulated in the same direction in CB1(–/–) mice compared with WT controls (dotted bars). Only genes with significant expression changes (ANOVA  $P < 0.05$ ) are shown.

CB1 receptor inverse agonist is more potent in food intake and body weight reduction in obese mice (Hildebrandt et al., 2003; Vickers et al., 2003). In addition, there was almost no overlap in the AM251 signatures in either WT or CB1(–/–) mice, ruling out the potential off-target effect in gene expression studies.

Our microarray data showed that AM251 decreased the expression of both SREBP-1 and SREBP-2, leading to the down-regulation of their target genes in de novo fatty acid and cholesterol synthesis. The down-regulation of SREBP-1 target genes is consistent with an earlier report that SR141716 [5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide], a CB1 inverse agonist, suppresses de novo fatty acid synthesis in liver. Indeed, the decrease of SREBP-1c, FASN, and ACC1 mRNA was confirmed by Northern blot assays (Osei-Hyiaman et al., 2005). The simultaneous suppression of both de novo fatty acid and cholesterol synthesis pathways probably contributes to the known beneficial effects of CB1 inverse agonists in improving insulin sensitivity and cardio-metabolic risk factors in both human and rodent models (Després et al., 2005; Van Gaal et al., 2005; Pi-Sunyer et al., 2006; Scheen et al., 2006; Gary-Bobo et al., 2007; Addy et al., 2008; Osei-Hyiaman et al., 2008).

Chronic treatment of obese rodent models with CB1 inverse agonists induces sustained weight loss even though it causes less reduction in food intake after the second week of treatment (Ravinet Trillou et al., 2003; Doyon et al., 2006; Gary-Bobo et al., 2007; Serrano et al., 2008), suggesting a contribution of energy expenditure toward weight reduction. Indeed, several groups showed CB1 inverse agonists increase oxygen consumption and/or reduce respiratory quotient in both rodent models (Liu et al., 2005; Herling et al., 2007; Flamment et al., 2009) and human models (Addy et al., 2008). We detected a significant increase in the expression of genes involved in fatty acid  $\beta$ -oxidation in livers of DIO mice treated with AM251, possibly mediated through PPAR $\alpha$  activation. Osei-Hyiaman et al. (2008) demonstrated that CB1 inverse agonism increases CPT1a mRNA, protein, and enzymatic activity, suggesting transcriptional regulation of this pathway translates into physiological effects.

The effect of adipose CB1 receptor signaling is less validated than liver, although there is evidence suggesting CB1



receptor antagonists/inverse agonists induce adiponectin expression (Bensaid et al., 2003), suppress lipogenesis (Cota et al., 2003), and promote mitochondrial biogenesis (Tedesco et al., 2008) in adipose tissue or cultured adipocytes. Studies of respiratory quotient and total energy expenditure in WT, global, and liver-specific CB1 receptor knockout mice clearly demonstrated an extrahepatic contribution toward energy expenditure (Osei-Hyiaman et al., 2008). Similar to the observation in liver, we saw down-regulation of the expression of genes involved in de novo fatty acid and cholesterol synthesis in eWAT, which is probably mediated through SREBPs. This observation aligns with earlier reports that treatment with CB1 inverse agonists leads to smaller adipocytes (Jbilo et al., 2005). We also noticed a modest up-regulation of genes involved in fatty acid  $\beta$ -oxidation and energy expenditure in eWAT (Fig. 7). The extent of the increase in eWAT was not as robust as in liver, probably because of the relative low content of mitochondria in white adipose tissue. Yet the observation indicates the roles of extra-hepatic tissues such as adipose in mediating energy expenditure by CB1 inverse agonists. Liu et al. (2005) reported that SR141716 increases oxygen consumption and glucose uptake in the soleus muscle of *ob/ob* mice. Although it is not clear whether this is mediated through direct action on muscle CB1 receptors or a secondary effect, the data support contribution of skeletal muscle toward increased energy expenditure induced by CB1 inverse agonist. We did not collect muscle samples in this experiment. The regulation of genes and pathways in muscle should be investigated in future studies.

Our observation that AM251 caused a down-regulation in the TNF $\alpha$  signaling pathway in eWAT highlights a mechanism underlying improved insulin sensitivity by CB1 inverse agonists, given the well established association between inflammation and insulin resistance. Suppression of the TNF $\alpha$  pathway could lead to activation of PPAR $\gamma$  and downstream target genes. Although we were not able to detect a significant increase of PPAR $\gamma$  mRNA after a 2-day treatment with AM251, many of its target genes involved in lipid uptake and lipogenesis were up-regulated. Although activation of PPAR $\gamma$  clearly improves insulin sensitivity, it could also lead to increased adipocyte differentiation in adipose tissue. This may sound paradoxical given the fact that CB1 inverse agonists decrease adiposity. We speculate that the potential adipocyte differentiation effect of PPAR $\gamma$  activation can be counteracted by suppression of SREBPs and increase of energy expenditure in both liver and adipose tissue. Our hypothesis of activation of PPAR $\gamma$  target genes is also supported by the report that adiponectin is increased by CB1 inverse agonist treatment (Bensaid et al., 2003; Jbilo et al., 2005). We were unable to detect significant regulation of adiponectin mRNA, probably because of the relatively short treatment of AM251 in our experiments.

Restriction of food intake was reported to suppress SREBP target genes and increase fatty acid  $\beta$ -oxidation pathways in liver or adipose tissues (Désert et al., 2008; Nishikawa et al., 2008; Nogueiras et al., 2008; Wang et al., 2009). In our experiment, the animals were not pair-fed, and there was a significant food intake difference between AM251 and vehicle treatment, thus one can not exclude the contribution of hypophagia toward the regulation of de novo fatty acid synthesis and fatty acid  $\beta$ -oxidation pathways. However, we believe that the reduction of food intake only partially accounts for the observed effect. In the CB1(–/–) mice versus WT com-

parison, many of the gene regulations observed by AM251 treatment were also detected in CB1(–/–) compared with WT mice, and this is unlikely to have been caused entirely by hypophagia because the food intake effect in CB1(–/–) mice is more subtle than the effect of AM251 treatment on food intake.

Our studies demonstrated a profound effect of AM251 in peripheral tissues, with the liver gene expression changes also confirmed by Agilent ink-jet oligonucleotide arrays (data not shown), a microarray platform (Hughes et al., 2001) different from the Affymetrix chips used in this article. AM251 is a brain-penetrating compound, thus we cannot exclude the impact of CNS on peripheral tissues. Nogueiras et al. (2008) attempted to address the central versus peripheral effect of CB1 inverse agonist by comparing intracerebroventricular and intraperitoneal administration of rimonabant (SR141716) in DIO rats. They observed an increase of glucose uptake and expression of CPT1a and a decrease of SCD1 in adipose tissues that can not be explained by a reduction of food intake in only the intraperitoneal treatment. Although their results were consistent with the contribution of peripheral effect toward increased energy expenditure and glucose homeostasis, the possibility that the intraperitoneally administered inverse agonist reaches populations of brain CB1 receptors that are not reached by intracerebroventricular administration cannot be ruled out. Ideally the central versus peripheral mode of action of CB1 inverse agonists should be addressed by profiling of a non-brain-penetrating compound and validating it in tissue-specific CB1 KO models. Although gene expression profiling alone is not sufficient to reveal the mechanism of CB1 inhibition leading to improved insulin sensitivity and metabolic parameters, our analysis sets the foundation for future hypothesis-driven validation.

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#### References

- Addy C, Wright H, Van Laere K, Gantz I, Erondun N, Musser BJ, Lu K, Yuan J, Sanabria-Bohórquez SM, Stoch A, et al. (2008) The acyclic CB1R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. *Cell Metab* 7:68–78.
- Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, and Soubrié P (2003) The cannabinoid CB1 receptor antagonist SR141716 increases Acp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 63:908–914.
- Berger J and Moller DE (2002) The mechanisms of action of PPARs. *Annu Rev Med* 53:409–435.
- Berger JP, Akiyama TE, and Meinke PT (2005) PPARs: therapeutic targets for metabolic disease. *Trends Pharmacol Sci* 26:244–251.
- Bloom SR, Kuhajda FP, Laher I, Pi-Sunyer X, Ronnett GV, Tan TM, and Weigle DS (2008) The obesity epidemic: pharmacological challenges. *Mol Interv* 8:82–98.
- Brown MS and Goldstein JL (1997) The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 89:331–340.
- Cota D, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thöne-Reineke C, Ortmann S, et al. (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 112:423–431.
- Désert C, Duclos MJ, Blavy P, Lecerf F, Moreews F, Klopp C, Aubry M, Herault F, Le Roy P, Berri C, et al. (2008) Transcriptome profiling of the feeding-to-fasting transition in chicken liver. *BMC Genomics* 9:611.
- Després JP, Golay A, Sjöström L, and Rimonabant in Obesity-Lipids Study Group (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* 353:2121–2134.
- Doyon C, Denis RG, Baraboi ED, Samson P, Lalonde J, Deshaies Y, and Richard D

- (2006) Effects of rimonabant (SR141716) on fasting-induced hypothalamic-pituitary-adrenal axis and neuronal activation in lean and obese Zucker rats. *Diabetes* **55**:3403–3410.
- Flamment M, Gueguen N, Wetterwald C, Simard G, Malthiery Y, and Ducluzeau PH (2009) Effects of the cannabinoid CB1 antagonist, rimonabant, on hepatic mitochondrial function in rats fed a high fat diet. *Am J Physiol Endocrinol Metab* **297**:E1162–E1170.
- Gary-Bobo M, Elachouri G, Gallas JF, Janiak P, Marini P, Ravinet-Trillou C, Chabbert M, Cruccioli N, Pfersdorff C, Roque C, et al. (2007) Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats. *Hepatology* **46**:122–129.
- Gatley SJ, Gifford AN, Volkow ND, Lan R, and Makriyannis A (1996) 123I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. *Eur J Pharmacol* **307**:331–338.
- Guilherme A, Virbasius JV, Puri V, and Czech MP (2008) Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* **9**:367–377.
- Hayden MS and Ghosh S (2008) Shared principles in NF- $\kappa$ B signaling. *Cell* **132**:344–362.
- Herling AW, Gossel M, Haschke G, Stengelin S, Kuhlmann J, Müller G, Schmolli D, and Kramer W (2007) CB1 receptor antagonist AVE1625 affects primarily metabolic parameters independently of reduced food intake in Wistar rats. *Am J Physiol Endocrinol Metab* **293**:E826–E832.
- Hildebrandt AL, Kelly-Sullivan DM, and Black SC (2003) Antiobesity effects of chronic cannabinoid CB1 receptor antagonist treatment in diet-induced obese mice. *Eur J Pharmacol* **462**:125–132.
- Hughes TR, Mao M, Jones AR, Burchard J, Marton MJ, Shannon KW, Lefkowitz SM, Ziman M, Schelter JM, Meyer MR, et al. (2001) Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat Biotechnol* **19**:342–347.
- Jbilo O, Ravinet-Trillou C, Arnone M, Buisson I, Bribes E, Péleraux A, Pénarier G, Soubrie P, Le Fur G, Galiegue S, et al. (2005) The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. *Faseb J* **19**:1567–1569.
- Jones D (2008) End of the line for cannabinoid receptor 1 as an anti-obesity target? *Nat Rev Drug Discov* **7**:961–962.
- Kelly LJ, Vicario PP, Thompson GM, Candelore MR, Doebber TW, Ventre J, Wu MS, Meurer R, Forrest MJ, Conner MW, et al. (1998) Peroxisome proliferator-activated receptors  $\gamma$  and  $\alpha$  mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. *Endocrinology* **139**:4920–4927.
- Kunos G, Osei-Hyiaman D, Bátkai S, Sharkey KA, and Makriyannis A (2009) Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci* **30**:1–7.
- Lee GY, Kim NH, Zhao ZS, Cha BS, and Kim YS (2004) Peroxisomal-proliferator-activated receptor  $\alpha$  activates transcription of the rat hepatic malonyl-CoA decarboxylase gene: a key regulation of malonyl-CoA level. *Biochem J* **378**:983–990.
- Lefebvre P, Chinetti G, Fruchart JC, and Staels B (2006) Sorting out the roles of PPAR  $\alpha$  in energy metabolism and vascular homeostasis. *J Clin Invest* **116**:571–580.
- Liu YL, Connolly IP, Wilson CA, and Stock MJ (2005) Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. *Int J Obes (Lond)* **29**:183–187.
- Martin G, Schoonjans K, Lefebvre AM, Staels B, and Auwerx J (1997) Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPAR $\alpha$  and PPAR $\gamma$  activators. *J Biol Chem* **272**:28210–28217.
- Moller DE and Berger JP (2003) Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *Int J Obes Relat Metab Disord* **27**:S17–S21.
- Muise ES, Azzolina B, Kuo DW, El-Sherbeini M, Tan Y, Yuan X, Mu J, Thompson JR, Berger JP, and Wong KK (2008) Adipose fibroblast growth factor 21 is up-regulated by peroxisome proliferator-activated receptor  $\gamma$  and altered metabolic states. *Mol Pharmacol* **74**:403–412.
- Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, Pang Z, Chen AS, Ruderman NB, Chen H, et al. (2006) Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor  $\gamma$  agonists. *J Biol Chem* **281**:2654–2660.
- Nishikawa S, Doi K, Nakayama H, and Uetsuka K (2008) The effect of fasting on hepatic lipid accumulation and transcriptional regulation of lipid metabolism differs between C57BL/6J and BALB/c mice fed a high-fat diet. *Toxicol Pathol* **36**:850–857.
- Nogueiras R, Veyrat-Durebex C, Suchanek PM, Klein M, Tschöp J, Caldwell C, Woods SC, Wittmann G, Watanabe M, Liposits Z, et al. (2008) Peripheral, but not central, CB1 antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. *Diabetes* **57**:2977–2991.
- Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Bátkai S, Harvey-White J, Mackie K, Offertaler L, Wang L, et al. (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* **115**:1298–1305.
- Osei-Hyiaman D, Liu J, Zhou L, Godlewski G, Harvey-White J, Jeong WI, Bátkai S, Marsicano G, Lutz B, Buettner C, et al. (2008) Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest* **118**:3160–3169.
- Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J, and RIO-North America Study Group (2006) Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA* **295**:761–775.
- Rakhshandehroo M, Sanderson LM, Matilainen M, Stienstra R, Carlberg C, de Groot PJ, Müller M, and Kersten S (2007) Comprehensive analysis of PPAR $\alpha$ -dependent regulation of hepatic lipid metabolism by expression profiling. *PPAR Res* **2007**:26839.
- Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, and Soubrie P (2003) Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* **284**:R345–R353.
- Reddy JK and Rao MS (2006) Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. *Am J Physiol Gastrointest Liver Physiol* **290**:G852–G858.
- Ruan H, Hacoen H, Golub TR, Van Parijs L, and Lodish HF (2002a) Tumor necrosis factor- $\alpha$  suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor- $\kappa$ B activation by TNF- $\alpha$  is obligatory. *Diabetes* **51**:1319–1336.
- Ruan H, Miles PD, Ladd CM, Ross K, Golub TR, Olefsky JM, and Lodish HF (2002b) Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor- $\alpha$ : implications for insulin resistance. *Diabetes* **51**:3176–3188.
- Ruderman NB, Saha AK, Vavvas D, and Witters LA (1999) Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol Endocrinol Metab* **276**:E1–E18.
- Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF, and RIO-Diabetes Study Group (2006) Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet* **368**:1660–1672.
- Serrano A, Del Arco I, Javier Pavón F, Macías M, Perez-Valero V, and Rodríguez de Fonseca F (2008) The cannabinoid CB1 receptor antagonist SR141716A (Rimonabant) enhances the metabolic benefits of long-term treatment with oleoylethanolamide in Zucker rats. *Neuropharmacology* **54**:226–234.
- Sfeir Z, Ibrahim A, Amri E, Grimaldi P, and Abumrad N (1997) Regulation of FAT/CD36 gene expression: further evidence in support of a role of the protein in fatty acid binding/transport. *Prostaglandins Leukot Essent Fatty Acids* **57**:17–21.
- Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, and Goldstein JL (1997) Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* **99**:846–854.
- Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, et al. (1999) Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J Biol Chem* **274**:35832–35839.
- Tedesco L, Valerio A, Cervino C, Cardile A, Pagano C, Vettor R, Pasquali R, Carruba MO, Marsicano G, Lutz B, et al. (2008) Cannabinoid type 1 receptor blockade promotes mitochondrial biogenesis through endothelial nitric oxide synthase expression in white adipocytes. *Diabetes* **57**:2028–2036.
- Tontonoz P, Hu E, Devine J, Beale EG, and Spiegelman BM (1995) PPAR $\gamma$ 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* **15**:351–357.
- Van der Ploeg LH (2000) Obesity: an epidemic in need of therapeutics. *Curr Opin Chem Biol* **4**:452–460.
- Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rössner S, and RIO-Europe Study Group (2005) 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* **365**:1389–1397.
- Vickers SP, Webster LJ, Wyatt A, Dourish CT, and Kennett GA (2003) Preferential effects of the cannabinoid CB1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. *Psychopharmacology (Berl)* **167**:103–111.
- Wang PH, Ko YH, Chin HJ, Hsu C, Ding ST, and Chen CY (2009) The effect of feed restriction on expression of hepatic lipogenic genes in broiler chickens and the function of SREBP1. *Comp Biochem Physiol B Biochem Mol Biol* **153**:327–331.
- Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, et al. (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* **8**:1288–1295.
- Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM, and Moller DE (1996) Negative regulation of peroxisome proliferator-activated receptor- $\gamma$  gene expression contributes to the antiadipogenic effects of tumor necrosis factor- $\alpha$ . *Mol Endocrinol* **10**:1457–1466.
- Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, and Bonner TI (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA* **96**:5780–5785.

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