A peripheral endocannabinoid mechanism contributes to glucocorticoid-mediated metabolic syndrome

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Glucocorticoids are known to promote the development of metabolic syndrome through the modulation of both feeding pathways and metabolic processes; however, the precise mechanisms of these effects are not well-understood. Recent evidence shows that glucocorticoids possess the ability to increase endocannabinoid signaling, which is known to regulate appetite, energy balance, and metabolic processes through both central and peripheral pathways. The aim of this study was to determine the role of endocannabinoid signaling in glucocorticoid-mediated obesity and metabolic syndrome. Using a mouse model of excess corticosterone exposure, we found that the ability of glucocorticoids to increase adiposity, weight gain, hormonal dysregulation, hepatic steatosis, and dyslipidemia was reduced or reversed in mice lacking the cannabinoid CB_1 receptor as well as mice treated with the global CB_1 receptor antagonist AM251. Similarly, a neutral, peripherally restricted CB_1 receptor antagonist (AM6545) was able to attenuate the metabolic phenotype caused by chronic corticosterone, suggesting a peripheral mechanism for these effects. Biochemical analyses showed that chronic excess glucocorticoid exposure produced a significant increase in hepatic and circulating levels of the endocannabinoid anandamide, whereas no effect was observed in the hypothalamus. To test the role of the liver, specific and exclusive deletion of hepatic CB_1 receptor resulted in a rescue of the dyslipidemic effects of glucocorticoid exposure, while not affecting the obesity phenotype or the elevations in insulin and leptin. Together, these data indicate that glucocorticoids recruit peripheral endocannabinoid signaling to promote metabolic dysregulation, with hepatic endocannabinoid signaling being especially important for changes in lipid metabolism.

corticosterone | 2-AG | anandamide | obesity | liver

Obesity and associated cardiometabolic diseases, such as type 2 diabetes, represent major contributors to morbidity and mortality (1, 2). Persistent exposure to environmental and psychological stress and the concomitant increase in circulating glucocorticoids (GCs; the primary stress hormones) are believed to be contributing factors to the epidemic of obesity and metabolic syndrome (3–6). Furthermore, patients receiving exogenous GCs subsequent to an organ transplant or for the treatment of inflammatory-related illnesses show several symptoms of the metabolic syndrome (7). Despite this relationship, the mechanisms by which GCs produce these changes in weight regulation and metabolism remain unclear.

It has been clearly shown that GCs can mobilize the endocannabinoid (eCB) system, which is essential for many of the effects of GCs, including negative feedback regulation of the hypothalamic–pituitary–adrenal axis, suppression of sexual behavior, and alterations in memory consolidation (8–11). eCBs are also potent regulators of feeding and metabolism, with effects that parallel those of GCs, such as increased feeding, reduced energy expenditure, fat accumulation within the liver, dyslipidemia, and development of adiposity and obesity (12–16). These observations led us to hypothesize that long-term exposure to elevations in GCs results in a hyperactive eCB system, which contributes to metabolic syndrome and obesity. Using a combination of genetic and pharmacological tools to ablate the eCB system, we show that eCB signaling through the CB_1 receptor (CB_1R) within the periphery contributes to the development of obesity and metabolic syndrome in a mouse model of excess GC exposure. Moreover, these effects seem independent of central feeding mechanisms. In this study, we use our group's recently characterized noninvasive approach to deliver a high dose of corticosterone (CORT; 100 μg/mL) in the drinking water to mice, resulting in the rapid development of a metabolic syndrome phenotype (14). This model mirrors clinical symptoms observed in chronic CORT treatment (such as after organ transplantation or for the treatment of inflammatory disease). Our findings substantiate the role of the eCB system in obesity and metabolic syndrome and extend these findings to show an

Significance

Obesity and associated metabolic disorders (e.g., cardiovascular disease and type 2 diabetes) are major public health concerns. These disorders result, in part, from hormonal dysregulation, particularly of glucocorticoids (GCs; central regulators of metabolism and adipogenesis). The specific mechanisms by which GCs modulate these processes remain largely unknown, but GCs increase production of endocannabinoids—potent central and peripheral regulators of appetite, energy balance, and metabolism. Our results show that sustained exposure to GCs produces obesity and metabolic syndrome through a peripheral endocannabinoid mechanism. These data further our understanding of the role of endocannabinoid signaling to promote not only diet-induced, but also, hormonal-mediated obesity and support the argument that peripheral blockade of endocannabinoid signaling could be a potential treatment for obese conditions.

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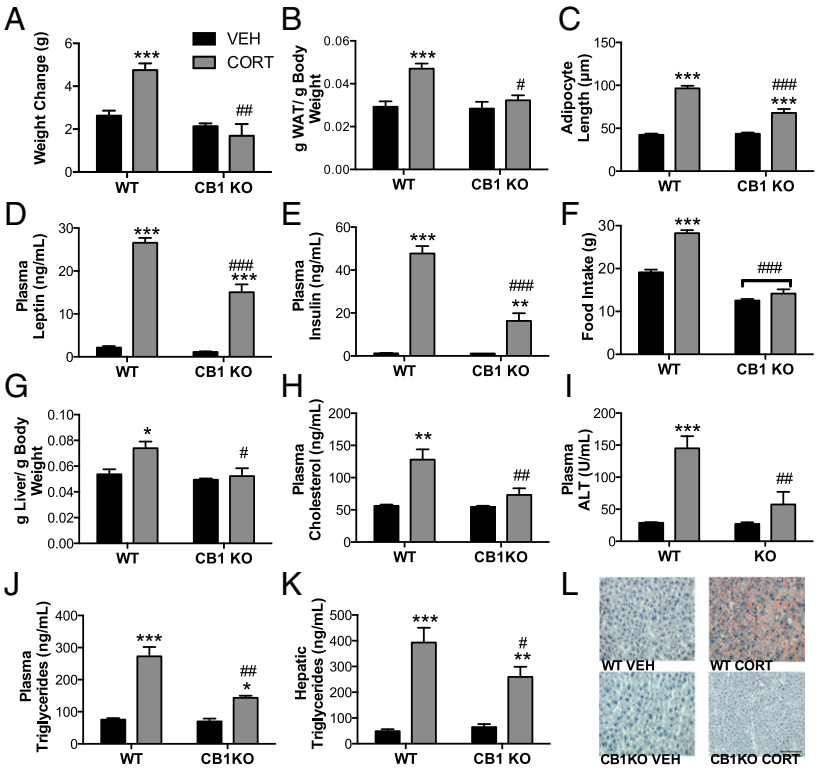

Fig. 1. CB_1R signaling is required for GC-mediated metabolic abnormalities. Graphs show that CORT-treated $CB_1R^{-/-}$ mice have reduced (*A*) weight, (*B*) adiposity, and (*C*) adipocyte size. Similarly, (*D*) plasma insulin and (*E*) plasma leptin as measured by ELISA show a blunted CORT-induced increase compared with WT. (*F*) The CORT-induced increase in food consumption is reduced in $CB_1R^{-/-}$ mice as shown in week 4 of food intake; however, pair-feeding studies show that this hyperphagia does not mediate the development of obesity (Fig. S2). $CB_1R^{-/-}$ mice are also protected against the CORT-induced increase in (*G*) liver weight, (*H*) plasma cholesterol, (*I*) alanine aminotransferase (ALT), (*J*) triglycerides, and (*K*) hepatic triglycerides. (*L*) $CB_1R^{-/-}$ mice are also protected against the development of hepatic steatosis as noted by the decreased accumulation of lipid droplets in the liver as measured by Oil Red O staining as well as decreased macrovesicular steatosis as measured by H&E staining (Fig. S1*B*). Data are expressed as means ± SEMs (n = 4–5 per group). Asterisks indicate the significant effects of CORT treatment relative to vehicle treatment in mice. Pound signs indicate statistically significant differences between CORT-treated WT and $CB_1R^{-/-}$ mice. VEH, vehicle. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. (Scale bar, 100 μm.)

important role of eCB signaling in hormonally mediated obesity. This pattern of results supports the development of peripherally restricted CB_1R antagonists as possible therapeutics for these conditions.

Results

Metabolic Assessment of Mice Treated with CORT. Consistent with previous data from our group (14, 17), mice exposed to CORT rapidly developed a metabolic syndrome phenotype characterized by significant increases in body weight, adiposity, hyperlipidemia, hyperleptinemia, hyperglycemia, hyperinsulinemia, hyperphagia, hepatic steatosis, and an array of other metabolic measures (Fig. 1 and Fig. S1). As long-term GC exposure has been shown to stimulate hunger and increase the rewarding aspects of food intake (18–21), we conducted a pair-feeding study to further explore if hyperphagia contributed to the obesity phenotype shown herein. CORT-treated animals became comparably obese, even when the amount of food was restricted to that consumed by untreated mice (Fig. S2); thus, the ability of CORT to induce obesity does not require hyperphagia and is likely the result of changes in metabolism. To probe this possibility, we determined the respiratory quotient (RQ) in CORT-treated mice as a measure of fuel-partitioning patterns. Mice exposed to chronic CORT exhibit a range from 0.91 to 0.97 compared with from 0.77 to 0.95 in vehicle-treated mice, suggesting a preference for carbohydrate oxidation and an inability to fluctuate between carbohydrate oxidation and fatty acid oxidation (Fig. S3).

Genetic and Pharmacological Evidence That CORT-Induced Metabolic Dysregulation Is CB_1R-Dependent. To elucidate the role of CB_1R signaling in metabolic dysregulation, global CB_1R KO ($CB_1R^{-/-}$) mice and their WT littermates were exposed to the drinking water CORT model for 28 d. Genetic ablation of the CB_1R significantly attenuated or completely abolished the effects of chronic CORT on the metabolic measures (Fig. 1 and Fig. S1). Circulating CORT levels and the degree of splenic atrophy induced by CORT were comparable in WT and $CB_1R^{-/-}$ mice, supporting the notion that differences in the CORT effect in WT and $CB_1R^{-/-}$ mice were not caused by differences in exposure to CORT (Fig. S4). CORT-treated $CB_1R^{-/-}$ mice had an RQ range of 0.87–0.96 resulting from greater fatty acid oxidation compared with WT CORT mice (RQ range = 0.91–0.97) (Fig. S3). Together, these data support the hypothesis that CB_1R-mediated signaling is required for metabolic dysregulation induced by chronic GC exposure.

To further establish the role of the eCB system in GC-induced metabolic syndrome, WT mice were treated with daily injections of the global CB_1R antagonist AM251 (2 mg/kg) throughout the 28-d CORT treatment. Consistent with the effects seen in $CB_1R^{-/-}$ mice, AM251 treatment significantly attenuated CORT-induced increases in body weight, adiposity, circulating leptin, and insulin (Fig. 2). AM251 treatment also resulted in a reduction of CORT-stimulated fat accumulation in the liver (Fig. 2*F*), confirming the hypothesis that CB_1R signaling is necessary for the development of CORT-induced obesity and metabolic alterations. These data are consistent with findings that CB_1R signaling is elevated in obese individuals and functionally contributes to the development of metabolic syndrome (13).

Alterations in the Central and Peripheral eCB Systems in CORT-Treated Mice. Metabolic function in several organ systems is modulated by eCB signaling (13). Within the brain, hypothalamic eCB signaling stimulates increased energy intake, whereas in the liver and white adipose tissue (WAT), eCB signaling contributes to various metabolic components of obesity (13, 22). To localize eCB effects in tissues important for metabolic regulation, the eCBs 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA) as well as the related fatty acid ethanolamides *N*-oleoylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA) were quantified by MS in the hypothalamus, blood, WAT, and liver (Table 1). CORT treatment did not alter the content of AEA or PEA in the hypothalamus but decreased the amount of OEA and 2-AG, supporting the argument against a central site of action for enhanced eCB

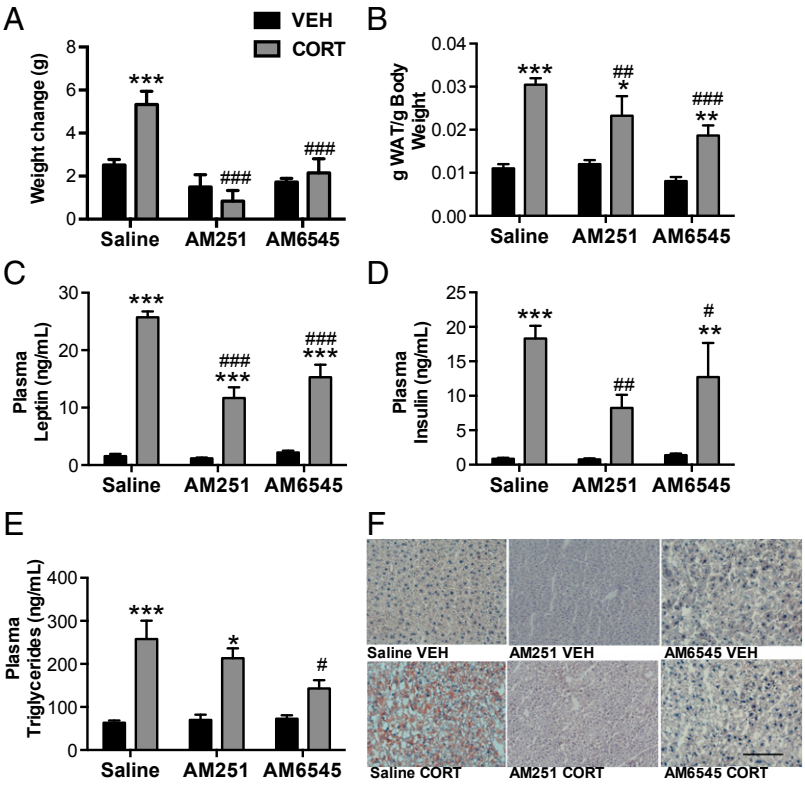

Fig. 2. Blockade of the CB_1R modulates the effect of chronic CORT. Concurrent treatment of CORT in the water for 28 d with either the global CB_1R antagonist AM251 or the peripheral specific CB_1R antagonist AM6545 results in decreased (*A*) weight, (*B*) adiposity, (*C*) circulating plasma leptin, and (*D*) insulin compared with WT controls. (*E*) Triglycerides were significantly decreased in AM6545-treated mice but remained unaltered in AM251-treated mice. (*F*) However, both AM251 and AM6545 treatments prevent development of hepatic steatosis as indicated by Oil Red O staining. Data are expressed as means ± SEMs ($n = 4$–9 per group). Asterisks indicate the significant effects of CORT treatment relative to vehicle treatment in mice. Pound signs indicate the effect of AM251 or AM6545 compared with saline in CORT-treated mice. VEH, vehicle. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. (Scale bar, 100 μm.)

driving the CORT-mediated metabolic phenotype through an increase in feeding (23).

The liver and WAT are the primary organ systems identified as being responsible for the regulation of metabolism by eCB signaling (22, 24). CORT exposure nearly doubled hepatic AEA compared with vehicle-treated controls. Liver PEA content was similarly increased, but both OEA and 2-AG contents were significantly reduced by chronic CORT exposure. Although the decline in 2-AG content was somewhat unexpected, elevation in AEA content (without a concomitant increase in 2-AG) is consistent with results after DIO (25). CORT exposure significantly decreased AEA in the WAT and tended to decrease 2-AG (nearly reaching statistical significance), an effect mirroring animal models of diet-induced obesity (DIO) (26). In humans, circulating concentrations of these blood lipids positively relate to body mass index and obesity (16, 27), motivating us to examine circulating levels of eCBs for comparison. Chronic CORT treatment doubled plasma concentrations of AEA and increased concentrations of PEA and OEA as well, whereas plasma 2-AG concentrations were unaffected by CORT.

To further characterize the eCB system in these peripheral compartments, we examined the effects of CORT on gene expression of components of the eCB system within the liver and WAT. Specifically, we quantified mRNA for *N*-acyl-phosphatidylethanolamine–specific phospholipase D and fatty acid amide hydrolase (putative synthetic and catabolic enzymes, respectively) for AEA in liver and WAT (Table 2). Hepatic levels of *N*-acyl-phosphatidylethanolamine–specific phospholipase D mRNA were significantly increased, whereas fatty acid amide hydrolase mRNA levels were significantly reduced, consistent with increased liver contents of AEA and PEA. Additionally, mRNA for the CB_1R was increased within the liver after CORT treatment. There were no effects of CORT on the synthetic or catabolic enzymes responsible for 2-AG, diacylglycerol lipase-α, and monoacylglycerol lipase. Given the significant drop in 2-AG content, the lack of change in these catabolic enzymes is surprising but could be explained by alterations in enzymatic activity rather than expression levels. In WAT, the only significant effect of chronic CORT exposure was decreased expression of CB_1R mRNA. In whole, our data suggest that CORT exposure significantly increases hepatic AEA/CB_1R signaling, which was observed in DIO (22, 25), leading to the hypothesis that liver CB_1R signaling is necessary for chronic CORT to produce symptoms of metabolic disorder.

Peripheral CB_1R Antagonism Prevents Metabolic Dysregulation. Our data showing elevation in both circulating and hepatic AEA levels implicate a role for eCB signaling in peripheral compartments. To dissociate the central and peripheral effects of CB_1R signaling, we used the peripherally restricted, neutral (i.e., does not exhibit inverse agonist activity) CB_1R antagonist AM6545 (10 mg/kg) at a dose shown to exclusively occupy peripheral CB_1Rs (with no brain penetrance) and prevent the metabolic effects of DIO (28). Concurrent treatment with AM6545 and CORT resulted in blockade of CORT-induced weight gain, a significant decrease in CORT-induced adiposity, and blunting of elevations in circulating levels of leptin, insulin, and triglycerides (Fig. 2). AM6545 also prevented the development of hepatic steatosis relative to vehicle-treated animals exposed to CORT.

Liver-Specific Deletion of CB_1R Prevents CORT-Mediated Dyslipidemia but Not Obesity. Based on the results of our physiological, histological, and biochemical data, localizing the peripheral organ responsible for mediating the effects of eCB signaling on CORT-induced metabolic effects was important. Hepatocyte-specific $CB_1R^{-/-}$ ($LCB_1R^{-/-}$) mice are resistant to DIO-induced lipid dysregulation (22, 29, 30) and provide a powerful tool to explore involvement of liver eCB signaling. $LCB_1R^{-/-}$ mice revealed a unique role of liver eCB signaling in the metabolic effects of chronic CORT. The effects of chronic CORT on body weight, leptin, and insulin were not different between WT and $LCB_1R^{-/-}$ mice. However, the $LCB_1R^{-/-}$ mice exhibited significantly reduced effects of CORT on all measures of dyslipidemia, including plasma

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Table 1. The effects of chronic CORT administration on eCB levels

Tissue	AEA	2-AG	PEA	OEA
Hypothalamus tissue (pmol/g)				
Vehicle	6.74 ± 0.39	$4.46 \times 10^4 \pm 4.9$	222.56 ± 19.93	109.98 ± 6.49
CORT	6.05 ± 0.64	$3.17 \times 10^4 \pm 2.5$*	214.62 ± 4.87	90.04 ± 2.9*
Blood serum (pmol/mL)				
Vehicle	5.82 ± 0.920	8.55 ± 1.26	22.74 ± 2.67	8.23 ± 0.43
CORT	12.129 ± 2.737*	9.72 ± 2.14	39.05 ± 6.38*	10.17 ± 0.82*
WAT tissue (pmol/g)				
Vehicle	9.68 ± 0.97	2,008 ± 634	788.9 ± 116.3	301.2 ± 30.18
CORT	5.93 ± 0.39†	800.05 ± 95.99	590.0 ± 148.3	216.5 ± 40.57
Liver tissue(pmol/g)				
Vehicle	4.51 ± 0.86	840 ± 70	389.76 ± 29.56	70.19 ± 8.45
CORT	8.23 ± 1.10†	270 ± 20‡	503.72 ± 37.12*	32.85 ± 3.01†

Data are expressed as means ± SEMs. For eCB measures, $n = 4$–5 per group for hypothalamus, and $n = 8$–10 per group for blood, WAT, and liver.
*Significant difference at $P < 0.05$.
†Significant difference at $P < 0.01$.
‡Significant difference at $P < 0.001$.

triglyceride and alanine aminotransferase (ALT) concentrations, elevated liver weight, and hepatic steatosis (Fig. 3). The pattern of effects seen in the $LCB_1R^{-/-}$ mice is largely consistent with the protection against the hepatic effects of DIO afforded by loss of CB1R in hepatocytes (22). We conclude that enhanced hepatic AEA/CB_1R signaling mediates the dyslipidemic component of GC-induced metabolic syndrome: nonhepatic CB_1R signaling processes elsewhere in the periphery, such as adipose tissue, muscle, kidney, or pancreas (all of which have been shown to play some role in the metabolic effects of eCB signaling), mediate the effects of CORT on adiposity, hyperleptinemia, and hyperinsulinemia.

Discussion

Weight gain accompanies chronic stress exposure in many humans, and obesity is often associated with changes in the regulation of GC (3, 31–33). Although some (32, 34) but not all (35) studies indicate that adrenalectomy can prevent weight gain in rodent models of obesity, few animal models recapitulate the effects of hypercortisolemia on metabolic function. Our model reliably produces rapid and significant changes in body weight, feeding, and metabolic function in response to excess GC treatment, allowing for the investigation of the mechanisms by which GCs modulate these processes. This study shows that the CB_1R is necessary for the development of most of the metabolic effects of excessive GC hormone exposure. Our data indicate a significant role for hepatic CB_1R signaling in some of these effects, showing an important role of hepatocytes in the dyslipidemic effects of long-term hypercortisolemia. This study builds on the established obesogenic function of eCB signaling in response to dietary fat consumption by extending eCB involvement to hormonal mediators of metabolic dysregulation.

From our data, we also postulate an interaction between GCs and eCBs in the effects of DIO. Given that some studies have shown that DIO is dependent on both GC levels (32) and eCB signaling (36), our findings lead us to posit that GCs may be the mediating factor in mobilizing eCB signaling after high-fat feeding. Given our results and the findings of others (37), it seems likely that these two signaling systems function cooperatively to promote the development of metabolic syndrome. This data is consistent with data from other physiological systems, whereby many effects of GCs are mediated by increased eCB signaling (8). The mechanisms by which GCs increase eCB signaling are not well-understood, and different mechanisms seem to act in different tissues. Within the brain, evidence for both nongenomic and genomic (38–41) means for GC-induced eCB signaling exists. Our data suggest that, at least within the liver, these effects are likely mediated by genomic mechanisms, because sustained exposure to CORT resulted in changes in gene expression of many components of the eCB system that mapped onto functional changes in AEA levels and CB_1R signaling. More so, these data are consistent with reports in humans that circulating cortisol levels positively correlate with circulating levels of both AEA and PEA (42, 43). The specific mechanism of these interactions requires additional study, but our data support the overarching hypothesis that cross-talk between GC and eCB signaling is an important relationship for many physiological processes, including metabolic function.

Table 2. The effects of chronic CORT administration on mRNA of eCB parameters

Tissue	CB1R	FAAH	NAPE-PLD	MAGL	DAGL
WAT fold change					
Vehicle	1.84 ± 0.35	1.12 ± 0.28	1.10 ± 2.4	1.11 ± 0.36	1.11 ± 0.26
CORT	0.48 ± 0.16*	0.33 ± 0.14*	0.92 ± 0.18	0.66 ± 0.24	0.86 ± 0.30
Liver fold change					
Vehicle	1.01 ± 0.11	1.07 ± 0.09	0.73 ± 0.19	1.02 ± 0.05	1.46 ± 0.67
CORT	1.32 ± 0.03*	0.41 ± 0.016†	3.35 ± 0.37‡	1.41 ± 0.30	1.16 ± 0.39

Data are expressed as means ± SEMs ($n = 3$–4 per group). DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acyl-phosphatidylethanolamine–specific phospholipase D.
*Significant difference at $P < 0.05$.
†Significant difference at $P < 0.01$.
‡Significant difference at $P < 0.001$.

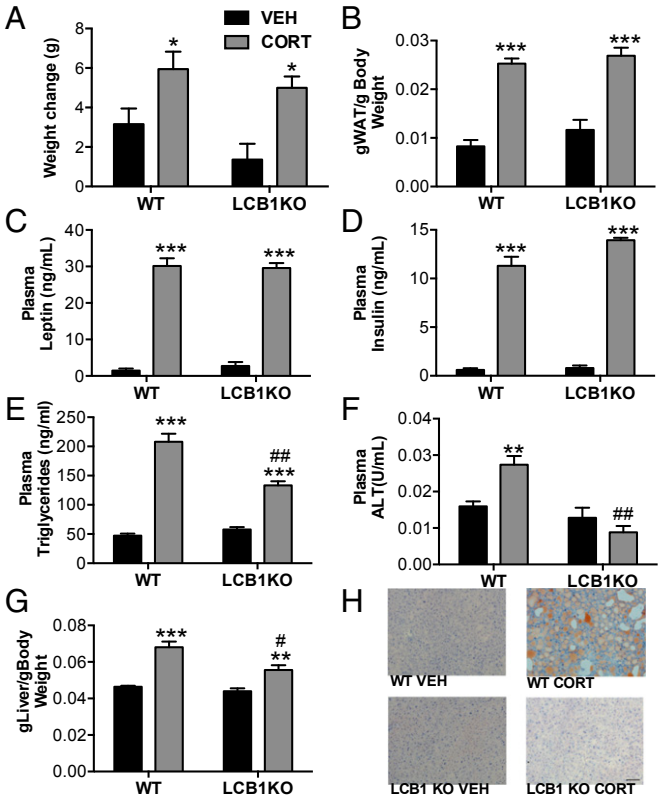

Fig. 3. Liver-specific $CB_1R^{-/-}$ reveals a unique role of hepatic signaling in CORT-treated mice. $LCB_1R^{-/-}$ does not prevent the CORT-induced (*A*) weight gain, (*B*) increased adiposity, or increased (*C*) plasma leptin and (*D*) insulin. In contrast, $LCB_1R^{-/-}$ does attenuate CORT-induced increases in (*E*) plasma triglycerides, (*F*) plasma alanine aminotransferase (ALT), and (*G*) hepatic triglycerides. (*H*) Furthermore, liver-specific KO of CB_1R prevents development of hepatic steatosis as indicated by Oil Red O staining. Data are expressed as means ± SEMs (n = 6–8 per group). Asterisks indicate the significant effects of CORT treatment relative to vehicle treatment in mice. Pound signs indicate statistically significant differences between CORT-treated WT and $LCB_1R^{-/-}$ mice. VEH, vehicle. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$. (Scale bar, 100 μm.)

The ability of eCB signaling to regulate metabolic function is clearly a complex process that involves many sites of action (12, 13). There is substantial evidence that eCB signaling within the brain, particularly within the hypothalamus (as well as the nucleus accumbens and olfactory bulb) (23, 44–49), promotes feeding behavior and its regulation by satiety state. Similarly, there is experimental evidence for both central and peripheral sites of action by which eCB signaling can modulate metabolic processes (12, 13, 50, 51), particularly through the regulation of sympathetic tone in adipose tissue. Peripheral eCB signaling has been suggested to account for various aspects of metabolic dysregulation in numerous tissue depots. Hepatic eCB signaling has been shown to promote de novo hepatic lipogenesis, hepatic steatosis, and global dysregulation of lipid metabolism (22, 29, 30, 52), whereas preliminary evidence suggests that CB_1R signaling within adipose tissue is important for the development of obesity and insulin resistance (53). Consistent with these findings, our data indicate that CB_1R within the liver drives the dyslipemic component of excess GC exposure, whereas blockade of all CB_1R in the periphery attenuates all aspects of the metabolic dysregulation. Based on the data from the adipose-specific $CB_1R^{-/-}$ mice (53), we predict that this tissue depot contributes significantly to many of the remaining metabolic effects of GCs that are not attributable to CB_1R within the liver. The exception here may be the residual leptin signaling produced by CORT treatment that was not reliably reversed by any form of CB_1R blockade or inactivation. Because there is evidence that CORT can directly drive leptin production in adipocytes (14, 54–57), the hyperleptinemic effects of GCs seem to be independent of CB_1R activity and are likely mediated by direct genomic actions of GCs. Future research will be required to examine this possibility.

Although their efficacy was promising in reducing the morbid effects of obesity, global CB_1R antagonists have been abandoned for the treatment of metabolic disorders because of centrally mediated psychiatric side effects (28). However, data from DIO models and our GC model indicate that eCB signaling seems to primarily mediate metabolic function through peripheral mechanisms. As such, peripherally restricted CB_1R antagonists may prove to be a valuable tool in the treatment of metabolic conditions without adverse psychiatric side effects, particularly those that are neutral antagonists and lack inverse agonist activity. Our data indicate the importance of peripheral eCB signaling in GC-mediated metabolic dysregulation and support the clinical investigation of peripherally restricted CB_1R in the treatment of both diet-induced and hormonally mediated forms of obesity.

Materials and Methods

Animals and Protocols. The *CB1R*$^{-/-}$ global and liver-specific mice used in this study were originally generated and backcrossed to a C57/Bl6J background (22). $LCB_1R^{-/-}$ mice were generated by crossing mice homozygous for the CB_1-floxed allele ($CB_1^{f/f}$), which were on a predominantly C57BL/6N background (seven to eight crossings), with mice expressing the bacterial Cre recombinase driven by the mouse albumin promoter (triglyceride[Alb-cre]′21 Mgn) that had been backcrossed seven times to a C57BL/6J background [Jackson Laboratory (58)] to obtain $CB_1^{f/f} \times CB_1^{f/fAlbCre}$ breeding pairs. All animal procedures were undertaken with approval of The Rockefeller University's Institutional Animal Care and Use Committee.

Pharmacological Manipulations. The general procedure for CORT-treated mice was conducted as previously described (14). Chronic treatment with AM251 [Tocris: DMSO; Tween 80: 0.9% saline 2:1:97 (vol/vol)] or AM6545 [DMSO; Tween 80: 0.9% saline 2:1:97 (vol/vol)] commenced concurrently with CORT in drinking water. One hour before lights off, mice were injected i.p. with vehicle [DMSO; Tween 80: 0.9% saline 2:1:97 (vol/vol)], AM251 at 2 mg/kg, or AM6545 at 10 mg/kg.

eCB Extraction and Analysis. Brain regions and liver samples were subjected to a lipid extraction process as described previously (59), and blood was analyzed as described elsewhere (60). eCB analysis for adipose tissue (61) is described in *SI Materials and Methods*. Oil Red O and H&E histology, pair feeding, quantitative RT-PCR (see Table S1 for a list of primers used), indirect calorimetery and derived metabolic measures (28), measurements of hepatic triglycerides, and measurements of very LDL triglyceride production are described in *SI Materials and Methods*.

Statistical Analyses. For all statistical analyses, we used Prism 5 (GraphPad Software, Inc.). Independent *t* test, one- or two-way ANOVA, or repeated measures ANOVAs were undertaken where appropriate. Our a priori hypothesis is that the *CB1R*$^{-/-}$ mice will be resistant to the effects of CORT administration, and therefore, regardless of significance of the interactions, Bonferroni posttests were used to examine differences in all variables among treatment conditions. In all cases, results were considered significant at $P < 0.05$. All statistical tests and results from these experiments can be seen in *SI Materials and Methods*.

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