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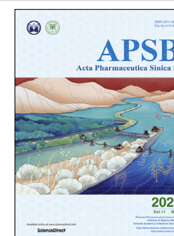

ORIGINAL ARTICLE

Gadd45b is required in part for the anti-obesity effect of constitutive androstane receptor (CAR)

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KEY WORDS

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Abstract Crosstalk between xenobiotic metabolism and energy metabolism in the liver has provided a potential opportunity to target xenobiotic receptors to treat metabolic diseases. Activation of constitutive androstane receptor (CAR), a xenobiotic-sensing nuclear receptor, has been shown to inhibit obesity, suppress hepatic gluconeogenesis, and ameliorate hyperglycemia in rodent models of obesity and type 2 diabetes. However, the underlying molecular mechanism remains to be defined. The growth arrest and DNA damage-inducible gene 45b (*Gadd45b*), a well-known anti-apoptotic factor, has been shown to be an inducible coactivator of CAR in promoting rapid liver growth. It is unknown whether the effect of CAR on energy metabolism depends on GADD45B. In the present study and by using a high fat diet (HFD)-induced obesity model, we show that reduced body weight gain and improved insulin sensitivity by the CAR agonist 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) were markedly blunted in *Gadd45b* knockout mice. Mechanistically, the TCPOBOP-responsive inhibition of hepatic lipogenesis, gluconeogenesis, and adipose inflammation observed in wild type mice were largely abolished in *Gadd45b* knockout mice. We conclude that *Gadd45b* is required in part for the metabolic benefits of CAR activation.

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1. Introduction

Constitutive androstane receptor (CAR) was initially characterized as a xenobiotic receptor in response to chemical exposures. The xenobiotic function of CAR was achieved through its transcriptional activation of drug metabolizing enzymes and transporters in liver and intestinal tracks[1−3].

The endobiotic function of CAR was subsequently appreciated, including its function in energy metabolism. For instance, CAR agonist phenobarbital has been anecdotally shown to have anti-obesity effects in humans[4], suggesting that CAR may play a role in energy metabolism. Several *in vitro* cell culture studies reported that activation of CAR decreased the mRNA expression of glucogenic enzyme genes *G6pase* and *Pepck*[5−8]. Finally, several *in vivo* studies have demonstrated the role of CAR in obesity and diabetes in mice[9,10]. Our group previously reported that activation of CAR improves insulin sensitivity in mice fed with high-fat diet (HFD), which was reasoned to be due to inhibition of lipogenesis, increased very low density lipoprotein (VLDL) secretion and energy expenditure[10]. Improved insulin sensitivity by CAR activation was also demonstrated by others using the leptin-deficient (*ob*/*ob*) mice[9]. At the mechanistic level, we recently reported that activation of CAR inhibits gluconeogenesis through accelerating degradation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-a), which is an essential coactivator in controlling glucose metabolism[7]. Dong et al.[9] showed that CAR activation attenuates hyperglycemia by suppressing glucose production and facilitating glucose uptake in the liver. A better understanding of the mechanisms underlying the metabolic benefits of CAR activation will help to harness the therapeutic potential of this “xenobiotic receptor”.

The growth arrest and DNA damage-inducible gene 45b (GADD45B) is an anti-apoptotic factor that can bind to mitogen-activated protein kinase kinase 7 (MKK7) and repress c-Jun N-terminal kinase (JNK) phosphorylation. Interestingly, GADD45B was later found to function as a coactivator for CAR[11,12]. Specifically, the hepatic expression of *Gadd45b* was found to be induced in the livers of mice treated with the CAR agonist TCPOBOP in a CAR-dependent and TNF-independent manner[13]. At the functional level, loss of *Gadd45b* impaired the early transcriptional stimulation caused by CAR activation, and *Gadd45b* is required to facilitate rapid liver growth[12]. However, whether *Gadd45b* is required for CAR-mediated improvement of insulin sensitivity and inhibition of lipogenesis and gluconeogenesis is yet to know.

In this study, we uncovered that *Gadd45b* is necessary for the metabolic benefits of CAR in inhibiting obesity and improving insulin sensitivity in mice.

2. Materials and methods

2.1. Animals

Wild type (WT) and *Gadd45b*$^{+/-}$ mice[14] in the C57BL/6J background were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). *Gadd45b*$^{-/-}$ knockout (KO) mice were generated by crossbreeding *Gadd45*b$^{+/-}$ mice (stock number: 013101). Genotyping primers for KO mice are 10936: GCAACCCCAGTAACTTTGGA; 10937: CCTGCAGGAGAGAAGGAGTG; oIMR7996: CTTCCATTTGTCACGTCCTG, provided by the Jackson Laboratory. Eight-week-old male WT and KO mice were subjected to 60% calories high fat diet (ENVIGO #TD.06414, containing 23.5% protein, 27.3% carbohydrate, and 34.3% fat, Harlan Laboratories, Madison, MI, USA) for 17 weeks. Mice received once per week intraperitoneal injections of 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP, 0.5 mg/kg) or vehicle (DMSO) as we have previously described[10]. Body composition was analyzed in live animals using EchoMRI-100TM from Echo Medical Systems (Houston, TX, USA). Mice were sacrificed 24 h after the last dose of drug. The use of animals in the study was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh (Pittsburgh, PA, USA).

2.2. Glucose tolerance test (GTT) and insulin tolerance test (ITT)

For GTT, mice were fasted for 16 h before receiving an intraperitoneal injection of D-glucose at 1.75 g/kg body weight. For ITT, mice were fasted for 6 h before receiving an intraperitoneal injection of human insulin (Novo Nordisk, Bagsvaerd, Denmark) at 1 unit/kg body weight. Blood samples were taken at different time points, and the concentrations of glucose were measured with a glucometer (OneTouch, Malvern, PA, USA). GTT and ITT were performed five days after the last TCPOBOP injection.

2.3. Liver triglyceride and cholesterol extraction and analysis

Liver lipids were extracted using chloroform−methanol method reported by Folch et al.[15]. Briefly, liver samples were homogenized in methanol first and chloroform was added to the sample. The whole mixture was agitated for 2 h at room temperature. Extra methanol was added, and the mixture was centrifuged at 1734×*g* for 5 min. Supernatant was collected and chloroform was added to the supernatant. After washing, the mixture was centrifuged at 771×*g* for 20 min and the bottom phase was collected and evaporated under nitrogen at 60 °C. The organic layer was reconstituted in a mixture of *tert*-butyl alcohol:Triton-114:methanol (4.5:2:1, *v*/*v*/*v*). Commercial assay kits from Stanbio (Boerne, TX, USA) were used to measure triglyceride and cholesterol levels.

2.4. Gene expression analysis

Total RNA was isolated using the TRIZOL (Invitrogen, Carlsbad, CA, USA) and treated with DNase I to remove genomic DNA. cDNA was synthesized using reverse-transcription kit (Thermo Fisher, Waltham, MA, USA). SYBR Green real time PCR was performed with the ABI 7500 real time PCR system (Waltham, MA, USA).

2.5. Histology

For H&E staining, tissues were harvested and fixed in 10% formalin overnight. After a dehydration process, tissues were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin. For oil red O staining, tissues were fixed in 1% paraformaldehyde for 3 h, dehydrated in 30% sucrose overnight at 4 °C and embedded in Tissue-Tek OCT compound (Fisher Scientific, Houston, TX, USA). Frozen liver tissues were cut at 8 μm and stained with oil red O solution (0.5% in isopropanol). Adipocyte size was quantified using Fiji Adiposoft software[16]

(Bethesda, MD, USA). Crown-like structures in white adipose tissue (WAT) were quantified using Image J software.

2.6. *Measurements of serum chemistry*

Serum levels of total triglyceride, cholesterol (Stanbio Laboratory, Boerne, TX, USA) and insulin (Crystal Chem, Downers Grove, IL, USA) were measured using commercial kits according to manufacturers' instructions.

2.7. *Statistical analysis*

Results are presented as means ± standard deviation (SD). Student's *t*-test was used for comparison between two groups. Analysis of variance (ANOVA) was used for the comparison among the means of three or more groups, followed by Tukey's post-test, using GraphPad PRISM software (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. *Gadd45b is required for the anti-obesity effect of CAR activation*

We have previously reported that treatment of mice with CAR agonist TCPOBOP alleviated HFD-induced obesity[10]. To determine whether the anti-obesity effect of CAR depends on *Gadd45b*, 8-week-old male WT and *Gadd45b* KO mice were fed with HFD for 17 weeks and weekly treated with TCPOBOP or vehicle. TCPOBOP significantly inhibited the gain of body weight as early as one week after the drug treatment in WT mice (Fig. 1A), but the inhibitory effect of TCPOBOP on body weight gain was largely abolished in KO mice (Fig. 1B). Body composition analysis by MRI show that after one week of TCPOBOP treatment in WT mice, the fat mass to body weight percentage was significantly higher in the vehicle group compared to the TCPOBOP group (Fig. 1C, top). Meanwhile, the lean mass to body weight percentage was decreased in the vehicle group, but they were steady in the TCPOBOP group (Fig. 1C, bottom). In contrast, neither the fat mass to body weight percentage (Fig. 1D, top) nor the lean mass to body weight percentage (Fig. 1D, bottom) was significantly different between the vehicle- and TCPOBOP-treated KO mice. The effect of *Gadd45b* on obesity was independent of changes in the food intake (Fig. 1E). At the end of week 17 treatment, the body weight gain (Fig. 1F) and fat mass to body weight percentage (Fig. 1G) of WT mice were significantly lower in the TCPOBOP group, while they were not significantly different between the vehicle and TCPOBOP groups in KO mice. The lean mass to body weight percentage was significantly higher in WT mice treated with TCPOBOP, but there was no difference between two groups in KO mice (Fig. 1H). Taken together, these results demonstrat that the anti-obesity effect by CAR activation was abolished in the *Gadd45b* knockout mice.

3.2. *Gadd45b is required for the insulin sensitizing effect of CAR activation*

Insulin resistance, or type 2 diabetes is one of the most prevalently metabolic complications closely associated with obesity[17]. Compared to lean subjects, the obese population is more likely to develop insulin resistance[18], which can be attenuated and even reversed by weight loss[19–21]. Consistent with our previous report[10], insulin sensitivity was significantly improved in WT mice treated with TCPOBOP, as shown by both the glucose tolerance test (Fig. 2A) and insulin tolerance test (Fig. 2B). However, TCPOBOP treatment was no longer effective in improving GTT (Fig. 2C) or ITT (Fig. 2D) performances in KO mice. Areas under the curve of GTT (Fig. 2E) and ITT (Fig. 2F) were significantly lower in TCPOBOP-treated WT mice, but not in TCPOBOP-treated KO mice. These results demonstrate that the insulin-sensitizing effect of CAR activation was also *Gadd45b* dependent.

At the biochemical level, analysis of serum biochemistry reveals that insulin level and fasting glucose level were significantly lower in TCPOBOP-treated WT mice but there is no difference between the vehicle group and the TCPOBOP treatment group in KO mice (Table 1). Interestingly, the serum triglyceride level was not affected by TCPOBOP in either WT or KO mice (Table 1). The serum cholesterol level was comparable between WT mice treated with or without TCPOBOP, but it was decreased by TCPOBOP in KO mice (Table 1).

3.3. *Gadd45b deficiency impairs TCPOBOP-responsive suppression of hepatic lipogenesis and lipogenesis, but has little effect on fatty acid β-oxidation*

As expected, the CAR target gene *Cyp2b10* was robustly induced by TCPOBOP in WT mice, and a similar degree of *Cyp2b10* induction was observed in KO mice (Fig. 3A). The intact induction of *Cyp2b10* by TCPOBOP in KO mice was consistent with the report that the attenuation of *Cyp2b10* induction by TCPOBOP in KO hepatocytes was early and transient[12]. Meanwhile, the mRNA expression of *Car* was reduced in TCPOBOP-treated WT mice but not in KO mice (Fig. 3B). Consistent with results published by others and us[10,22], treatment of WT mice with TCPOBOP alleviated HFD-induced hepatic steatosis as shown by oil red O staining of lipid droplets (Fig. 3C), or biochemical measurement of the liver triglyceride level (Fig. 3D). In HFD-fed KO mice, the basal oil red O staining (Fig. 3C) and liver triglyceride content (Fig. 3D) were significantly lower than their WT counterparts, and TCPOBOP was no longer effective in ameliorating hepatic steatosis. The liver cholesterol levels were not affected regardless of the *Gadd45b* genotype or the TCPOBOP treatment (Fig. 3E). The acetyl-coenzyme A carboxylase (ACC-1), the fatty acid synthase (FAS)[23], and the stearoyl-CoA desaturase (SCD-1)[24] are three key enzymes in the *de novo* lipogenesis in the liver. Treatment with TCPOBOP suppressed the expression of *Acc-1*, *Fas*, and *Scd-1* in WT mice, but not in KO mice (Fig. 3F). We next examined whether the expression of two upstream regulators of lipogenesis insulin-induced gene 1 protein (*Insig1*) and sterol regulatory element-binding protein 1 (*Srebp1-c*) was affected or not. The results show that *Insig1* mRNA expression was not changed in either WT or KO mice, whereas TCPOBOP treatment decreased *Srebp1-c* mRNA expression in both genotypes (Fig. 3G). Furthermore, the suppression of phosphoenolpyruvate carboxykinase (*Pepck*) and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (*Pgc1-a*) observed in TCPOBOP-treated WT mice was abolished in TCPOBOP-treated KO mice, but TCPOBOP remained effective in suppressing glucose-6-phosphatase (G6Pase) gene expression in KO mice (Fig. 3H). The suppression of peroxisome proliferator-activated receptor

alpha (*Ppara*) and its target genes involved in β-oxidation and fatty acid influx by TCPOBOP in WT mice was similarly observed in TCPOBOP-treated KO mice (Fig. 3I), suggesting that *Gadd45b* was not required for the suppression of fatty acid β-oxidation by CAR activation.

3.4. *Gadd45b deficiency abolishes the alleviation of inflammation in white adipose tissue (WAT) by TCPOBOP, but has little effect on the adipose lipogenesis and lipolysis*

Obesity is commonly associated with a state of chronic and low-grade inflammation that contributes to insulin resistance and type 2 diabetes[25,26]. Treatment of WT mice with TCPOBOP reduced adiposity in epididymal WAT, and this effect was abolished in TCPOBOP-treated KO mice, as shown by H&E staining (Fig. 4A) and quantification of adipocyte size (Fig. 4B). Adipose macrophage accumulation is higher in obesity, which is tightly related to insulin resistance[27,28]. Additionally, macrophage infiltration in the adipose tissue and liver also plays an important role in the pathogenesis of nonalcoholic fatty liver disease (NAFLD)[29]. TCPOBOP-treated WT mice showed a decreased number of macrophage-enriched crown-like structures (CLS) in their WAT, compared with vehicle-treated WT mice. In vehicle-treated KO mice, the basal density of CLS was decreased, and TCPOBOP was no longer effective in reducing the CLS density (Fig. 4A and C). The same pattern of *Gadd45b* knockout effect was observed when the adipose expression of macrophage marker genes *Cd68* and *F4/80* was measured (Fig. 4D). These results suggest that *Gadd45b* was essential for the effects of CAR activation on HFD-induced adipose tissue inflammation. The adipose expression of genes involved in lipogenesis (Fig. 4E) and lipolysis (Fig. 4F) was not affected by *Gadd45b* ablation.

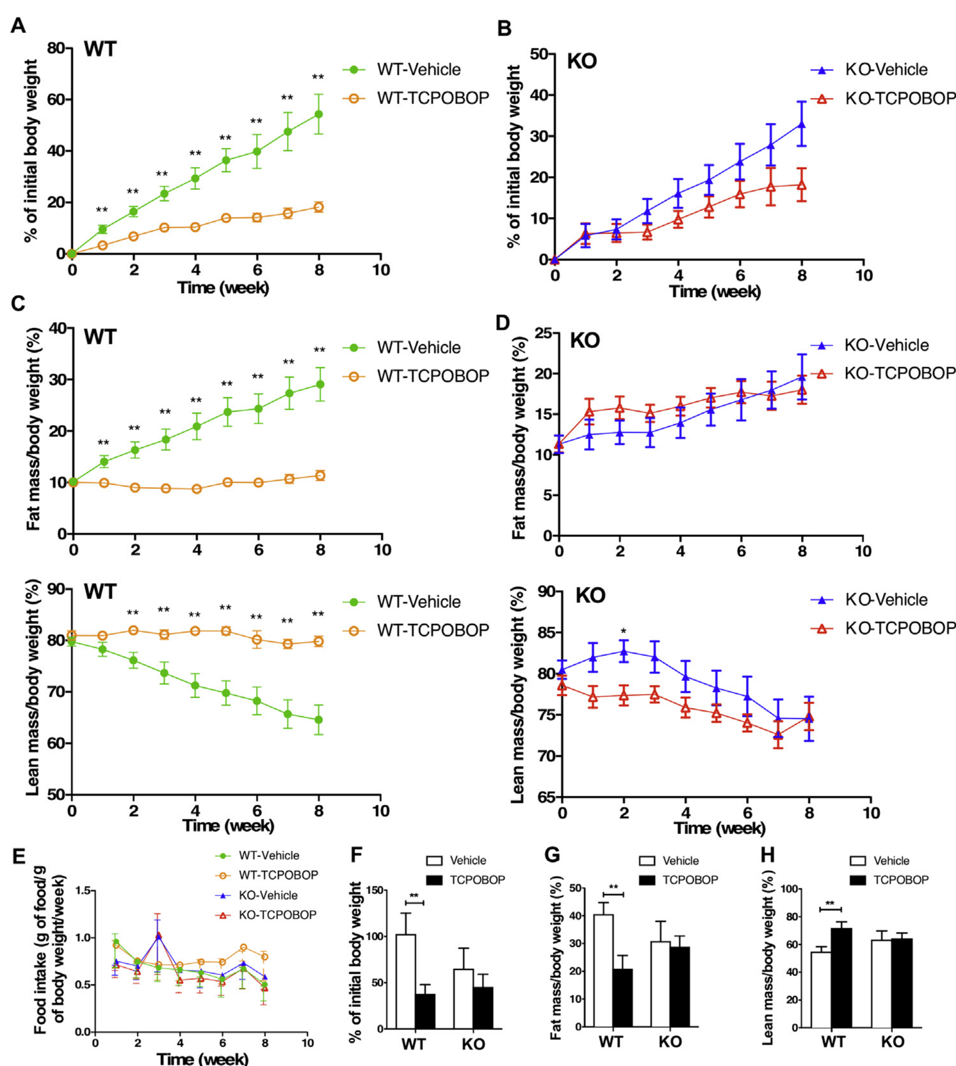

Figure 1 *Gadd45b* is required for the anti-obesity effect of CAR activation. Growth curve of male WT C57BL/6 mice (A) or *Gadd45b* KO mice (B) fed with HFD for 8 weeks, in the absence or presence of TCPOBOP treatment (0.5 mg/kg, intraperitoneal, once per week). Fat mass to body weight percentage (top) and lean mass to body weight percentage (bottom) in male WT C57BL/6 mice (C) or *Gadd45b* KO mice (D) fed with HFD for 8 weeks, in the absence or presence of TCPOBOP treatment (0.5 mg/kg, intraperitoneal, once per week). Fat mass and lean mass were determined by MRI, $n = 6$ for each group. (E) Food consumption of WT and KO mice fed with HFD treated with vehicle or TCPOBOP for 8 weeks. Body weight change (F), fat mass to body weight percentage (G), and lean mass to body weight (H) in male WT and KO C57BL/6 mice fed with HFD for 17 weeks. Results are presented as mean ± SD, $n = 6$ for each group. *$P < 0.05$; **$P < 0.01$ compared with the vehicle group.

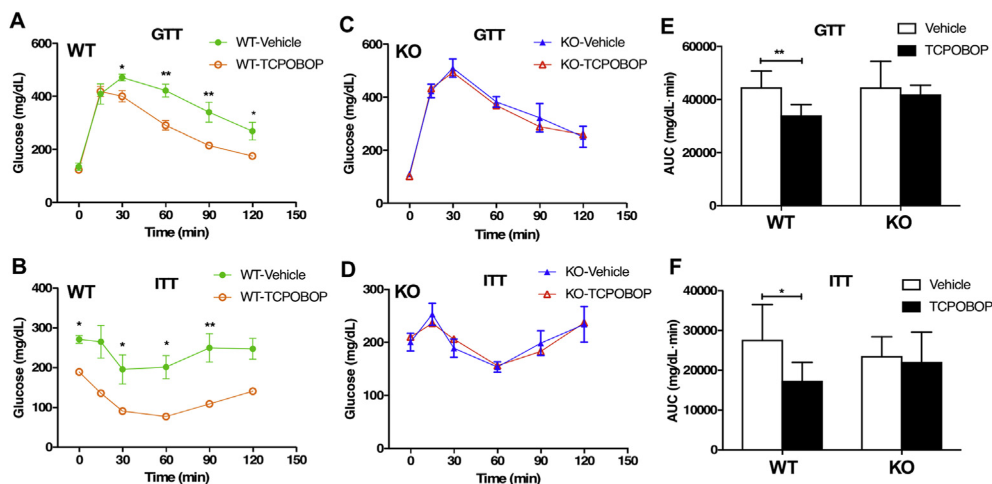

Figure 2 *Gadd45b* is required for the insulin sensitizing effect of CAR activation. Mice were fed with HFD for 8 weeks in the presence or absence of TCPOBOP (0.5 mg/kg, once per week) before glucose tolerance test (GTT, A) and insulin tolerance test (ITT). GTT (A) and ITT (B) in male WT C57BL/6J mice. GTT (C) and ITT (D) in male KO mice. Area under curve (AUC) of GTT (E) and ITT (F) in male WT and KO mice before GTT or ITT. Results are presented as mean ± SD, $n = 6$ for each group. *$P < 0.05$; **$P < 0.01$ compared with the vehicle group.

3.5. Gadd45b ablation has little effect on TCPOBOP-responsive attenuation of HFD-induced brown adipose tissue (BAT) whitening

Brown adipose tissue contains a large number of mitochondria, where fatty acid oxidation and heat emission take place. The whitening of brown adipose tissue was evident in obese animals[30]. We show that TCPOBOP treatment decreased the whitening of BAT in both WT and KO mice at the histological level (Fig. 5A), which indicated that *Gadd45b* may not be required for the effects of CAR activation on BAT. The *Gadd45b* genotype and HFD had little effect on the BAT expression of genes involved in lipogenesis (Fig. 5B) and β-oxidation (Fig. 5C).

4. Discussion

Gadd45b was originally known to be involved in cell death and proliferation[31]. *Gadd45b* was later found to be a gene inducible by CAR activation and was established as a co-activator of CAR. Activation of CAR is known to promote hepatocyte proliferation and cause hepatomegaly. It was found that loss of *Gadd45b* impairs the early transcriptional stimulation caused by CAR activation after partial hepatectomy, and *Gadd45b* is required to facilitate rapid liver growth[11,12]. CAR and its agonists such as TCPOBOP and phenobarbital are known to be potent tumor promoters in rodents and upon the diethylnitrosamine initiation[32,33]. Besides being a tumor promoter in rodents, TCPOBOP has also been shown to be a non-genotoxic hepatocarcinogen[34]. However, it has not been reported whether *Gadd45b* is required for the tumor promoting effect of CAR and its agonists. It is recognized that phenobarbital was not shown as a tumor promoter in humanized CAR mice[35]. It appeared that CAR-mediated liver carcinogenesis is rodent specific and upon diethylnitrosamine initiation, but it does not seem to be relevant for humans[36]. Phenobarbital was even reported to suppresses some liver tumors in certain context[37,38].

CAR is more recently known for its endobiotic functions, including its activity in attenuating obesity and type 2 diabetes. Several *in vitro* studies suggested that the inhibitory activities of CAR on lipogenesis and gluconeogenesis may have been due to the competitive binding of CAR to several transcription factors such as FOXO1, HNF4a, and PGC1a[5,39,40] onto the promoter regions of gluconeogenic or lipogenic genes. However, the *in vivo* significance of these transcriptional factors in mediating the metabolic benefits of CAR remains unclear. In this study, we demonstrated that as a CAR coactivator, *Gadd45b* is required for the anti-obesity and anti-diabetic effects of CAR *in vivo*. The improved systemic insulin sensitivity by CAR activation was abolished in KO mice. The serum insulin lowering effect of TCPOBOP was also abolished in *Gadd45b* KO mice. Loss of CAR-responsive suppression of hepatic lipogenesis and gluconeogenesis may have accounted for the loss of metabolic benefits of CAR activation in *Gadd45b* KO mice. Another interesting

Table 1 Serum chemistry in WT and KO mice fed with HFD for 17 weeks.

Index	WT		KO	
	Vehicle	TCPOBOP	Vehicle	TCPOBOP
Insulin (ng/mL)	4.088 ± 2.613	0.1454 ± 0.0528**	3.46 ± 2.031	2.075 ± 1.37
Triglyceride (mg/dL)	119.7 ± 22.06	111.6 ± 17.93	132.2 ± 35.88	99.85 ± 18.1
Cholesterol (mg/dL)	131.2 ± 16.09	117.4 ± 10.81	172.7 ± 43.11	118.9 ± 23.52*
Fasting glucose (mg/dL)	153.8 ± 17.63	126.0 ± 12.88**	114.5 ± 21.24	107.4 ± 21.88

Data are mean ± SD, $n = 6$. *$P < 0.05$, **$P < 0.01$ *vs.* Vehicle.

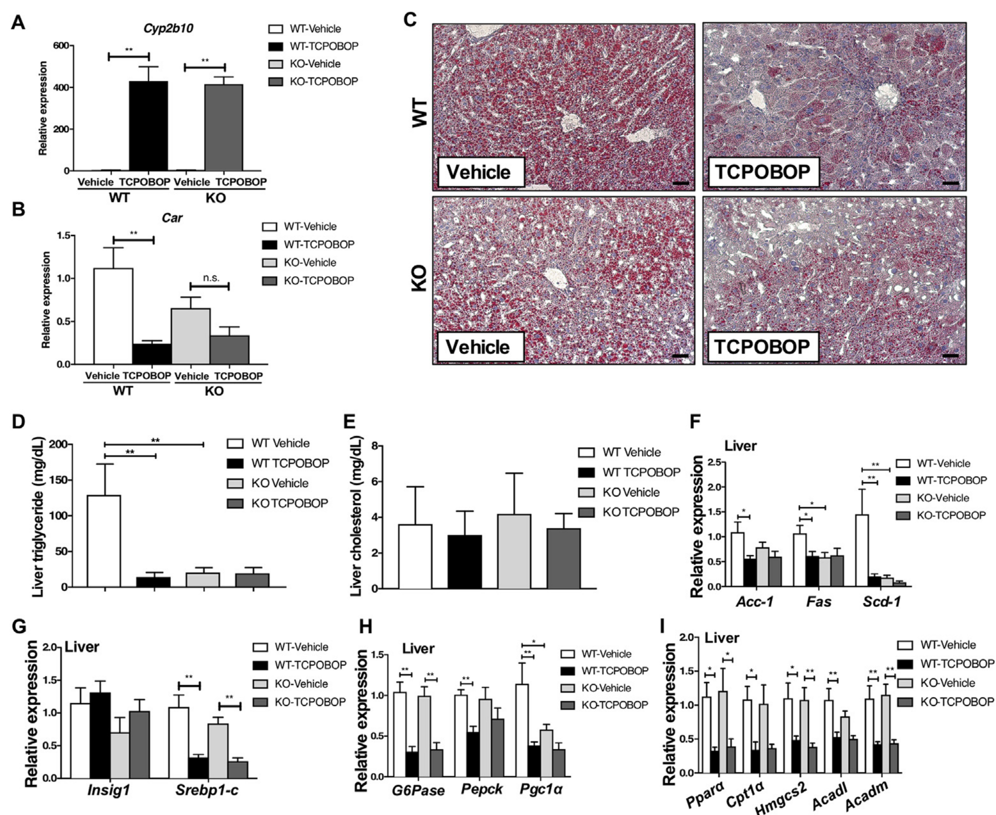

Figure 3 *Gadd45b* deficiency impairs TCPOBOP-responsive suppression of hepatic lipogenesis and lipogenesis, but has little effect on fatty acid β-oxidation. Mice were the same as described in Fig. 1. (A) and (B) The hepatic mRNA expression of *Cyp2b10* (A) and *Car* (B). (C) Hepatic steatosis was evaluated by oil red O staining. Scale bar, 50 μm. (D) and (E) Liver triglyceride (D) and cholesterol (E) levels. (F)−(I) The hepatic mRNA expression of genes involved in lipogenesis (F) and (G), gluconeogenesis (H), and β-oxidation (I) was measured by real-time PCR. Results are presented as mean ± SD, $n = 6$ for each group. *$P < 0.05$; **$P < 0.01$ compared with the vehicle group.

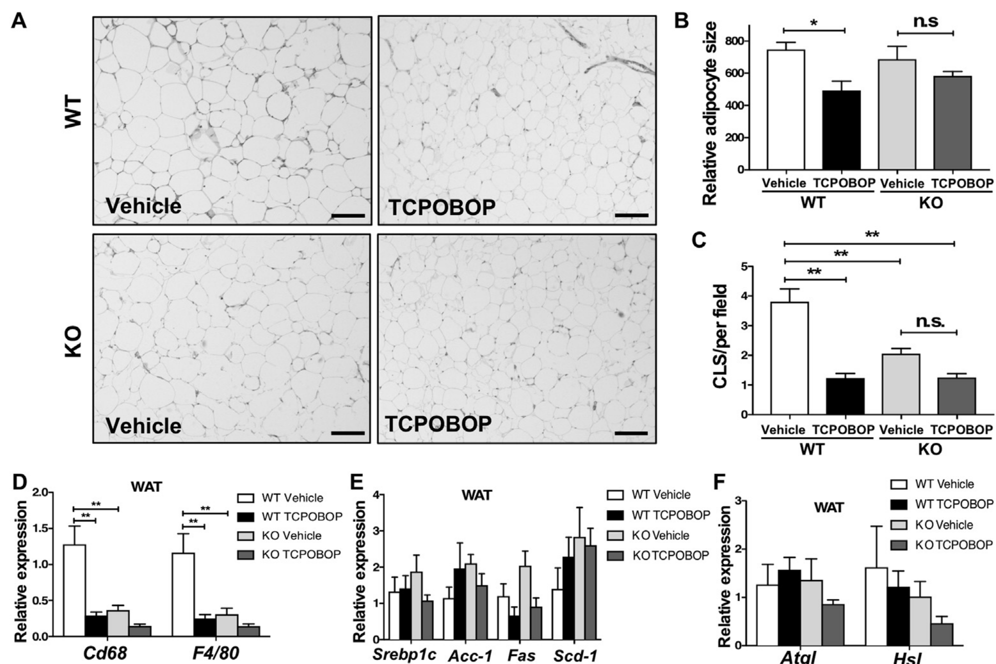

Figure 4 *Gadd45b* deficiency abolishes the alleviation of inflammation in white adipose tissue (WAT) by TCPOBOP, but has little effect on the adipose lipogenesis and lipolysis. Mice were the same as described in Fig. 1. (A) H&E staining of white adipose tissue (WAT). Scale bar = 100 μm. (B) Quantification of adipocyte size, and (C) quantification of crown-like structures (CLS). (D) The mRNA expression of macrophage marker genes and genes involved in (E) gluconeogenesis and (F) lipolysis in WAT was measured by real-time PCR. Results are presented as means ± SD, $n = 6$ for each group. *$P < 0.05$; **$P < 0.01$ compared with the vehicle group.

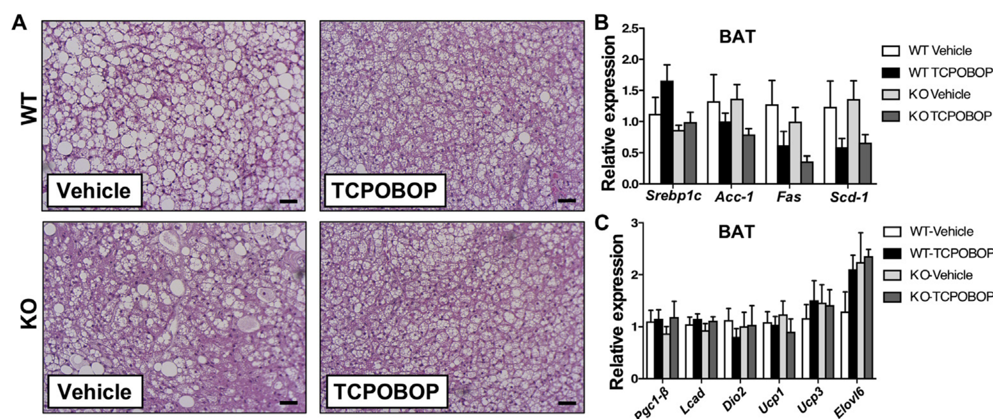

Figure 5 *Gadd45b* ablation has little effect on TCPOBOP-responsive attenuation of HFD-induced brown adipose tissue (BAT) whitening. Mice were the same as described in Fig. 1. (A) H&E staining of BAT. Scale bar, 50 μm. The mRNA expression of genes involved in lipogenesis (B) and energy expenditure and thermogenesis (C) was determined by real-time PCR. Results are presented as means ± SD, $n = 6$ for each group. $^{*}P < 0.05$; $^{**}P < 0.01$ compared with the vehicle group.

finding is that *Gadd45b* ablation abolished the alleviation of inflammation in WAT by TCPOBOP, which may have also contributed to the loss of metabolic benefits in KO mice. We have previously reported that WAT does not have appreciable expression of CAR[10]. As such, the WAT phenotype in TCPOBOP-treated KO mice was probably secondary to the loss of metabolic benefits in tissues outside of the adipose tissues.

Besides *Gadd45b*'s role in mediating the metabolic benefits TCPOBOP, we found that the vehicle-treated KO mice exhibited reduced liver triglyceride levels and decreased expression of lipogenic genes compared to their WT counterparts. The density of the CLS structure and the expression of adipose macrophage marker gene were also significantly decreased in vehicle-treated KO mice. These results suggested that *Gadd45b* may have CAR independent effects on energy metabolism. Since intestinal microbiota has been shown to play a vital role in HFD-induced obesity[41], we cannot exclude the possibility that changes in intestinal microbiota may have also contributed to the phenotypic exhibition.

Besides GADD45B, several other CAR coactivators of CAR, such as SRC1, SRC2, and PGC1a[42,43], have also been identified based on mammalian two-hybrid experiment and reporter gene assays. However, whether these coactivators contribute to the anti-obesity effects of CAR *in vivo* is not known. Meanwhile, we recently reported that *Gadd45b* can also function as a coactivator for another xenobiotic receptor aryl hydrocarbon receptor (AHR). Interestingly, *Gadd45b* was not required for the promotion of liver carcinogenesis by AHR activation[44].

There are several limitations of our study: 1) Mice of the C57BL background were used. It remains unclear whether the phenotype was strain dependent; 2) Only male mice were used. Although we have previously reported that the anti-obesity effect of TCPOBOP was not sex-specific[10], we cannot exclude the possibility that the phenotype of *Gadd45b* ablation was sex specific; and 3) The mechanism by which *Gadd45b* contributes to the metabolic benefits of CAR activation is yet to be defined. Previous reports have shown that CAR could compete with HNF4a for binding to the promoters of gluconeogenic genes[5], or facilitate the degradation of PGC1a[7]. It will be interesting to investigate whether *Gadd45b* affects the interaction between CAR and HNF4a or PGC1a, and thus conveying metabolic benefits.

In summary, we demonstrated that *Gadd45b* as a coactivator of CAR is required in part for the metabolic benefits of CAR activation *in vivo*.

Acknowledgment

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Author contributions

Xinran Cai, Ye Feng, and Wen Xie designed the study, analyzed the data, and drafted the original manuscript. Xinran Cai, Ye Feng, and Meishu Xu conducted the experiments. Xinran Cai, Ye Feng, Chaohui Yu, and Wen Xie reviewed and finalized the manuscript.

Conflicts of interest

Ye Feng was a visiting scholar at the University of Pittsburgh in the laboratory of Wen Xie from April 2017 to March 2019, during which Ye Feng was a graduate student of Zhejiang University First Affiliated Hospital (Hangzhou, China) under the supervision of Chaohui Yu. The authors declare no conflict of interest.

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