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Atypical antipsychotic drugs perturb AMPK-dependent regulation of hepatic lipid metabolism

Kyoung-Jin Oh,[1]* Jinyoung Park,[1]* Su Yeon Lee,[3] Injae Hwang,[4] Jae Bum Kim,[4] Tae-Sik Park,[3] Heon-Jeong Lee,[2] and Seung-Hoi Koo[1]

[1]*Department of Molecular Cell Biology and Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Gyeonggi-do;* [2]*Department of Psychiatry, Korea University College of Medicine, Seoul;* [3]*Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon; and* [4]*Department of Biophysics and Chemical Biology, School of Biological Sciences, Institute of Molecular Biology and Genetics, Seoul National University, Seoul, South Korea*

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Oh KJ, Park J, Lee SY, Hwang I, Kim JB, Park TS, Lee HJ, Koo SH. Atypical antipsychotic drugs perturb AMPK-dependent regulation of hepatic lipid metabolism. *Am J Physiol Endocrinol Metab* 300: E624–E632, 2011. First published January 11, 2011; doi:10.1152/ajpendo.00502.2010.—Dysregulation of lipid metabolism is a key feature of metabolic disorder related to side effects of antipsychotic drugs. Here, we investigated the molecular mechanism by which second-generation atypical antipsychotic drugs (AAPDs) affect hepatic lipid metabolism in liver. AAPDs augmented hepatic lipid accumulation by activating expression of sterol regulatory element-binding protein (SREBP) transcription factors, with subsequent induction of downstream target genes involved in lipid and cholesterol synthesis in hepatocytes. We confirmed the direct involvement of SREBPs on AAPD-induced expression of lipogenic and cholesterogenic genes by utilization of adenovirus for dominant negative SREBP (Ad-SREBP-DN). Interestingly, AAPDs significantly decreased phosphorylation of AMPKα and expression of fatty acid oxidation genes. Treatment of constitutive active AMPK restored AAPD-mediated dysregulation of genes involved in both lipid synthesis and fatty acid oxidation. Moreover, AAPDs decreased transcriptional activity of PPARα, a critical transcriptional regulator for controlling hepatic fatty acid oxidation, via an AMPK-dependent manner. Close investigations revealed that mutations at the known p38 MAPK phosphorylation sites (S6/12/21A), but not mutations at the putative AMPKα phosphorylation sites (S167/373/453A), block AAPD-dependent reduction of PPARα transcriptional activity, suggesting that p38 MAPK might be also involved in the regulatory pathway as a downstream effector of AAPDs/AMPK. Taken together, these data suggest that AAPD-stimulated hepatic dysregulation of lipid metabolism could result from the inhibition of AMPK activity, and pharmaceutical means to potentiate AMPK activity would contribute to restore hepatic lipid homeostasis that occurs during AAPD treatment.

adenosine 5′-monophosphate-activated protein kinase; sterol regulatory element-binding protein; peroxisome proliferator-activated receptor-α

ATYPICAL ANTIPSYCHOTIC DRUGS (AAPDs) have been used in the pharmacological treatment of schizophrenia (1). Although the second-generation AAPDs are much potent than the classical antipsychotics, treatment of AAPDs could cause troublesome side effects, such as obesity, insulin resistance, dyslipidemia, abnormal glucose tolerance, and diabetes (5, 11, 23, 36). Patients taking stable doses of clinically assigned olanzapine (OLZ) or clozapine (CLZ) have significantly higher fat mass, plasma insulin, glucose, triglyceride, and cholesterol levels (4, 5, 28). However, the exact molecular mechanism by which AAPDs lead to metabolic disorder remains unknown.

Dysregulation of lipid metabolism is a critical instigator for the metabolic disorder. Recently, it has been reported that antipsychotic drugs such as OLZ and CLZ activate maturation of sterol regulatory element-binding proteins (SREBPs) in cultured liver cells, glioma cells, and adipocytes (6, 33, 41). The SREBPs are major transcriptional regulators of cellular lipid and cholesterol synthesis (12, 14). Therefore, it is plausible to suspect that SREBP-mediated stimulation of lipogenesis might be crucial for antipsychotic drug-mediated dyslipidemia.

AMP-activated protein kinase (AMPK) plays a critical role in the regulation of hepatic energy metabolism (7, 31, 38). Activation of AMPK in the liver leads to the inhibition of lipogenesis as well as the stimulation of fatty acid oxidation. Inhibition of acetyl-CoA carboxylase (ACC) by AMPK reduces malonyl-CoA content, resulting in a subsequent decrease in lipid synthesis and an increase in mitochondrial fatty acid oxidation. In addition, AMPK has been shown to directly inhibit expression and/or activity of SREBP-1c (25, 31, 44, 48) and enhance activity of peroxisome proliferator-activated receptor-α (PPARα), a critical transcriptional factor for fatty acid oxidation (2, 45). Failure of a proper regulation of such pathways by AMPK may contribute to the development of nonalcoholic fatty liver disease. Indeed, treatment with the antidiabetic drug metformin reduces hepatic steatosis via activation of AMPK, suggesting a critical role of AMPK in the regulation of hepatic lipid homeostasis (24, 47).

Here, we report that increased expression of SREBPs is a key mechanism for AAPD-induced hepatic lipid synthesis. Furthermore, we show that AAPDs repressed AMPK activity to reduce PPARα-dependent fatty acid oxidation and to enhance SREBP-dependent lipid synthesis. These data support that AMPK activation would contribute to restore hepatic dysregulation of lipid metabolism that occurs during AAPD treatment.

MATERIALS AND METHODS

Drug preparation. For the cell culture studies, OLZ {LY-170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b][1,5]benzodiazepine; Zyprexa-Velotab; Eli Lilly} and CLZ (Sigma) were dissolved in DMSO at a final concentration of 50 mM. Cells were treated with

* These authors contributed equally to this work.
Address for reprint requests and other correspondence: S.-H. Koo, Dept. of Molecular Cell Biology and Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, 300 Chunchun-dong, Jangan-gu, Suwon, Gyeonggi-do, South Korea (e-mail: shkoo@skku.edu).

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25–50 μM OLZ or CLZ for 24–72 h. The concentration of drugs and the duration of the treatment were described in the figure legends in detail.

Animal experiments. Eight-week-old male C57BL/6 mice were purchased from Charles River Laboratories and housed in a specific pathogen-free facility at the Sungkyunkwan University School of Medicine (12:12-h light-dark cycle). CLZ was chronically self-administered in a small quantity of cookie dough, with 0.1 mg/g dough given to mice for 5 wk. Control mice received an equal quantity of drug-free dough. All procedures were approved by the Sungkyunkwan University School of Medicine Institutional Animal Care and Use Committee.

Isolation and culture of rat primary hepatocytes. Primary hepatocytes were prepared from 200 to 300 g of Sprague-Dawley rats by collagenase perfusion method as described (43). Cells were plated in medium 199 (Sigma) supplemented by 10% FBS, 10 U/ml penicillin, 10 mg/ml streptomycin, and 10 nM dexamethasone. After attachment, cells were infected with various adenoviruses and treated with drugs as indicated in the figure legends.

Plasmids and recombinant adenoviruses. PPARα mutants (S6/12/21A, S167/373/453A, and both) were generated using site-directed mutagenesis (44). Expression plasmid for SREBP Y320A [dominant negative SREBP (SREBP-DN), amino acids 1–403] (18) was purchased from Addgene (Addgene plasmid 8885). Adenoviruses expressing green fluorescent protein (GFP) only, 6X SRE-1 luc, SREBP Y320A (SREBP-DN, amino acids 1–403), and AMPKα T172D (AMPKα-CA, amino acids 1–312) were generated as described previously (20).

Oil Red O staining. Rat primary hepatocytes were washed twice with PBS. Monolayer cells were fixed on dishes with 3.7% formaldehyde in PBS for 30 min. Cells were stained with 40% isopropanol containing 0.5% Oil Red O for 1 h, followed by rinsing in 60% isopropanol and dH_2O to remove unbound dye. To quantify lipid accumulation, Oil Red O was extracted with 100% isopropanol, and the optical density of the solution was detected at 500 nm.

Total RNA preparation and quantitative PCR analysis. Total RNA from primary hepatocytes was extracted using an RNA extraction kit (Intron). Complementary DNA generated by reverse transcriptase

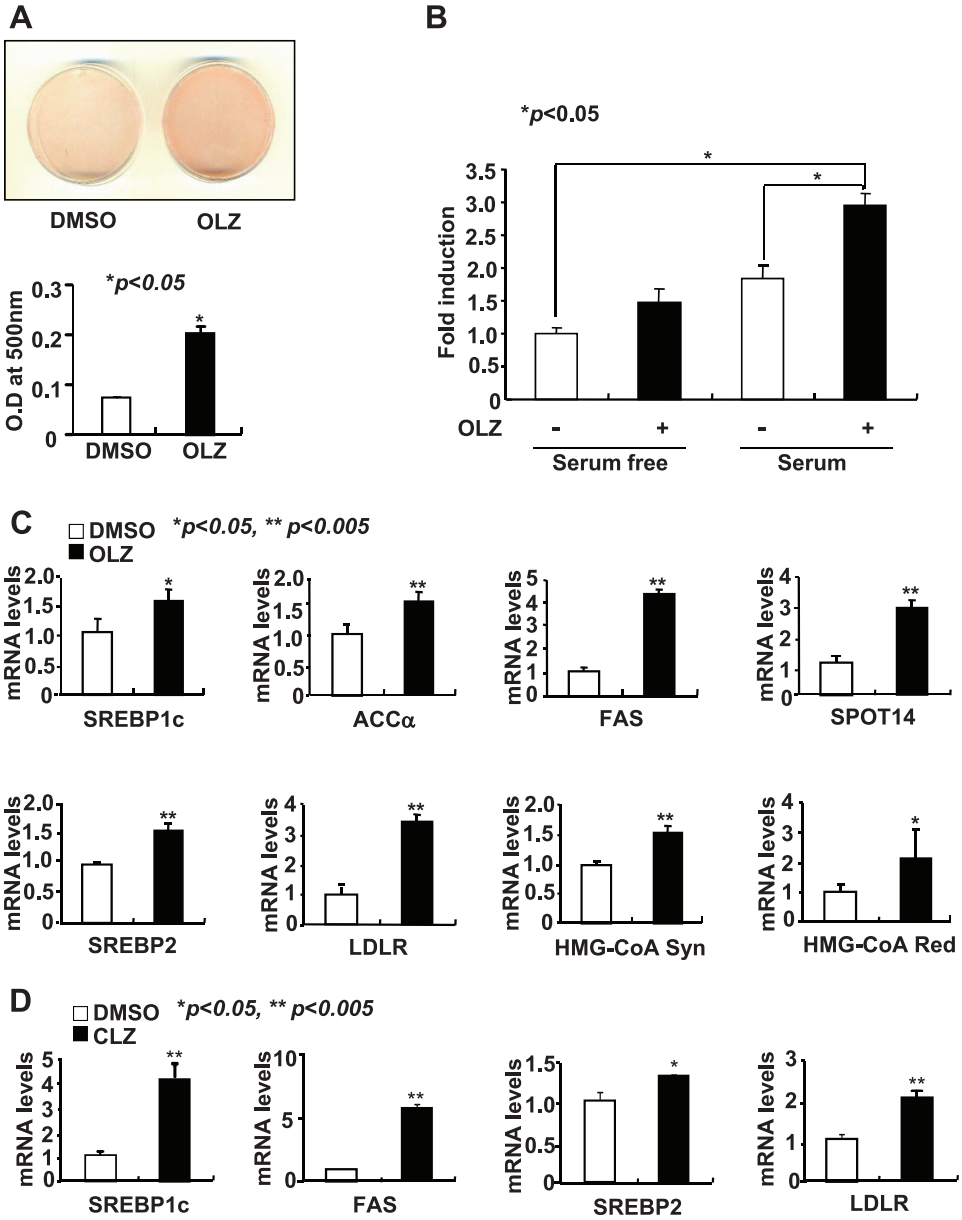

Fig. 1. Atypical antiphyschotic drugs (AAPDs) stimulate lipogenesis in primary hepatocytes. *A*: Oil Red O staining and optical density value showing hepatic lipid accumulation. Rat primary hepatocytes were treated with DMSO or 50 μM olanzapine (OLZ) for 72 h. *B*: OLZ-induced de novo lipogenesis in rat primary hepatocytes. *C* and *D*: quantitative PCR analysis showing effects of OLZ on expression of lipogenic [sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), acetyl-CoA carboxylase-α (ACCα), and SPOT14] and cholesterogenic [SREBP-2, LDL receptor (LDLR), hydroxymethylglutaryl coenzyme A reductase (HMG-CoA Red), and hydroxymethylglutaryl coenzyme A synthase (HMG-CoA Syn)] genes in rat primary hepatocytes. Cells were treated with DMSO, 25 μM OLZ (*C*), or 50 μM clozapine (CLZ; *D*) for 24 h. Data in *A–D* represent means ± SD.

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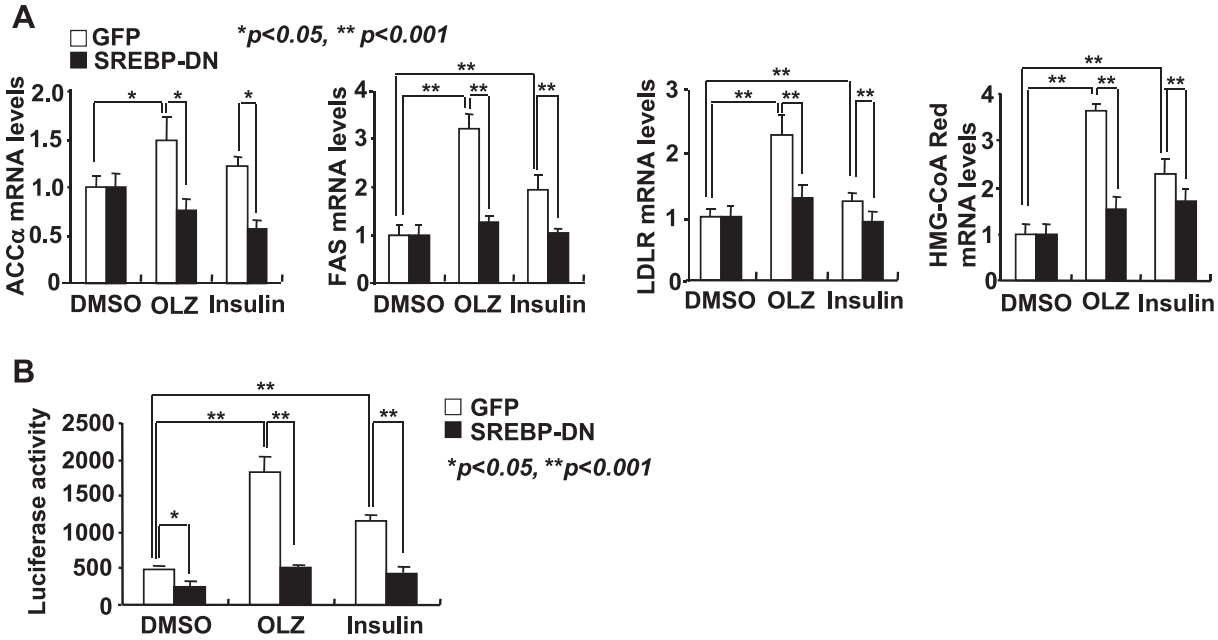

Fig. 2. AAPDs increase expression of lipid synthetic genes via SREBPs. *A*: quantitative PCR analysis showing effects of green fluorescent protein (GFP) or dominant negative SREBP (SREBP-DN) adenovirus on OLZ-dependent increase of lipogenic (ACCα and FAS) and cholesterogenic (LDLR and HMG-CoA reductase) gene expression. Rat primary hepatocytes were infected with adenovirus (Ad)-GFP or Ad-SREBP-DN and then treated with DMSO, 50 μM OLZ, or 100 nM insulin for 24 h. *B*: promoter assays showing effect of SREBP-DN on OLZ-induced Ad-SRE-1 luciferase activity in rat primary hepatocytes. Data represent means ± SD.

(GenDEPOT) was analyzed by quantitative PCR (QPCR) using a SYBR green PCR kit and a TP 800 Thermal Cycler Dice model (Takara) as described (21). Primers were designed using Oligoperfect Design (Invitrogen). The primers used for PCR are as follows: L32, forward 5′-TCTGGTGAAGCCCAAGATCG-3′ and reverse 5′-CTCTGGGTTTC-CGCCAGTT-3′; SREBP-1c, forward 5′-GTACCTGCGGGACAGCT-TAG-3′ and reverse 5′-TCAGGTCATGTTGGAAACCA-3′; SREBP-2, forward 5′-GCCTTCTGGAGACCATGGA-3′ and reverse 5′-

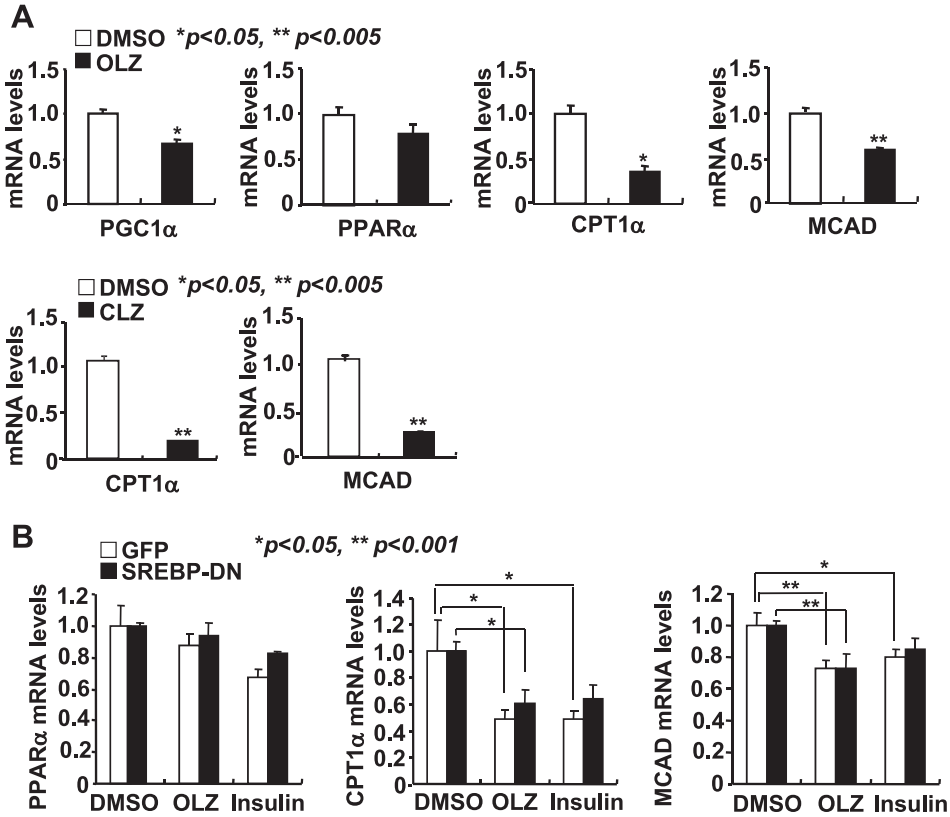

Fig. 3. AAPDs decrease expression of fatty acid oxidation genes. *A*: quantitative PCR analysis showing effects of AAPDs on expression of fatty acid oxidation genes [peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC1α), PPARα, carnitine palmitoyltransferase Iα (CPT Iα), and medium-chain acyl-CoA dehydrogenase (MCAD)]. Cells were treated with DMSO, 25 μM OLZ, or 50 μM CLZ for 24 h. *B*: quantitative PCR analysis showing effects of Ad-GFP or Ad-SREBP-DN on OLZ or insulin-reduced expression of fatty acid oxidation genes (PPARα, CPT Iα, and MCAD). Data represent means ± SD.

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ACAAAGTTGCTCTGAAAACAAATGA-3′; ACCα, forward 5′-GCGGGAGGAGTTCCTAATTC-3′ and reverse 5′-TGTCCCAGACG-TAAGCCTTC-3′; medium-chain acyl-CoA dehydrogenase (MCAD), forward 5′-GCTAGTGGAGCAGGAAGGAG-3′ and reverse 5′-CCTTCGCAATAGAGGCAAAG-3′; HMG-CoA reductase, forward 5′-CTTGTGGAATGCCTTGTGATTG-3′ and reverse 5′-AGC-CGAAGCAGCACATGAT-3′; fatty acid synthase (FAS), forward 5′-CGACCTCGCAGGGATTAACC-3′ and reverse 5′-TTCACGAATGGG-TAGCACCAG-3′; low-density lipoprotein receptor (LDLR), forward 5′-AGGCTGTGGGCTCCATAGG-3′ and reverse 5′-TGCGGTCCAGGG-TCATCT-3′; hydroxymethylglutaryl coenzyme A (HMG-CoA) synthase, forward 5′-GCCGTGAACTGGGTCGAA-3′ and reverse 5′-GCATA-TATAGCAATGTCTCCTG-3′;SPOT14, forward 5′-CCCAGCCTTCT-GAGGGATGT-3′ and reverse 5′-GCCCGTGGTCCACTTCTACA-3′; carnitine palmitoyltransferase Iα (CPT Iα), forward 5′-GTCTGAGCCATG-GAGGTTGT-3′ and reverse 5′-GGCTTGTCTCAAGTGCTTCC-3′; PPARα, forward 5′-AGAAGTTGCAGGAGGGGATT-3′ and reverse 5′-TCGGACTCGGTCTTCTTGAT-3′; and PPARγ coactivator-1α (PGC-1α), forward 5′-GCCTATGAGCACGAAAGGCT-3′ and reverse 5′-GCGCTCTTCAATTGCTTTCT-3′. All data were normalized to ribosomal L32 expression.

Western blot analysis. Western blot analyses on 10–60 μg of protein extracts from cells and liver tissue were performed as described (35). Antibodies for phospho-Ser^{79} ACC, ACC, phospho-Thr^{172} AMPKα, AMPKα, phospho-Thr^{389} p70 S6 kinase, p70 S6 kinase, and phospho-Thr$^{37/46}$ eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) were obtained from Cell Signaling Technology. heat shock protein 90α/β was used as a loading control, and its antibody was purchased from Santa Cruz Biotechnology.

Cell culture and transient transfection assays. Human hepatoma HepG2 cells were maintained with Ham's F-12 medium (cellgro) supplemented with 10% FBS, 10 U/ml penicillin, and 10 mg/ml streptomycin as described (35). For transfection, we used *Trans*IT-LT1 reagent (Mirus) with 300 [3X PPAR response element (PPRE): 5′-TGACCTXTGACCT-3]/150 ng (LXRE: 5′-AGGTCAXXXXAG-GTCA-3′) of luciferase construct, 50 ng of β-galactosidase plasmid, 2.5 ng of PPARα, and 100 ng of AMPKα2 T172D/1–5 ng of pcHA, pcMX, liver X receptor, and retinoid X receptor expression vectors.

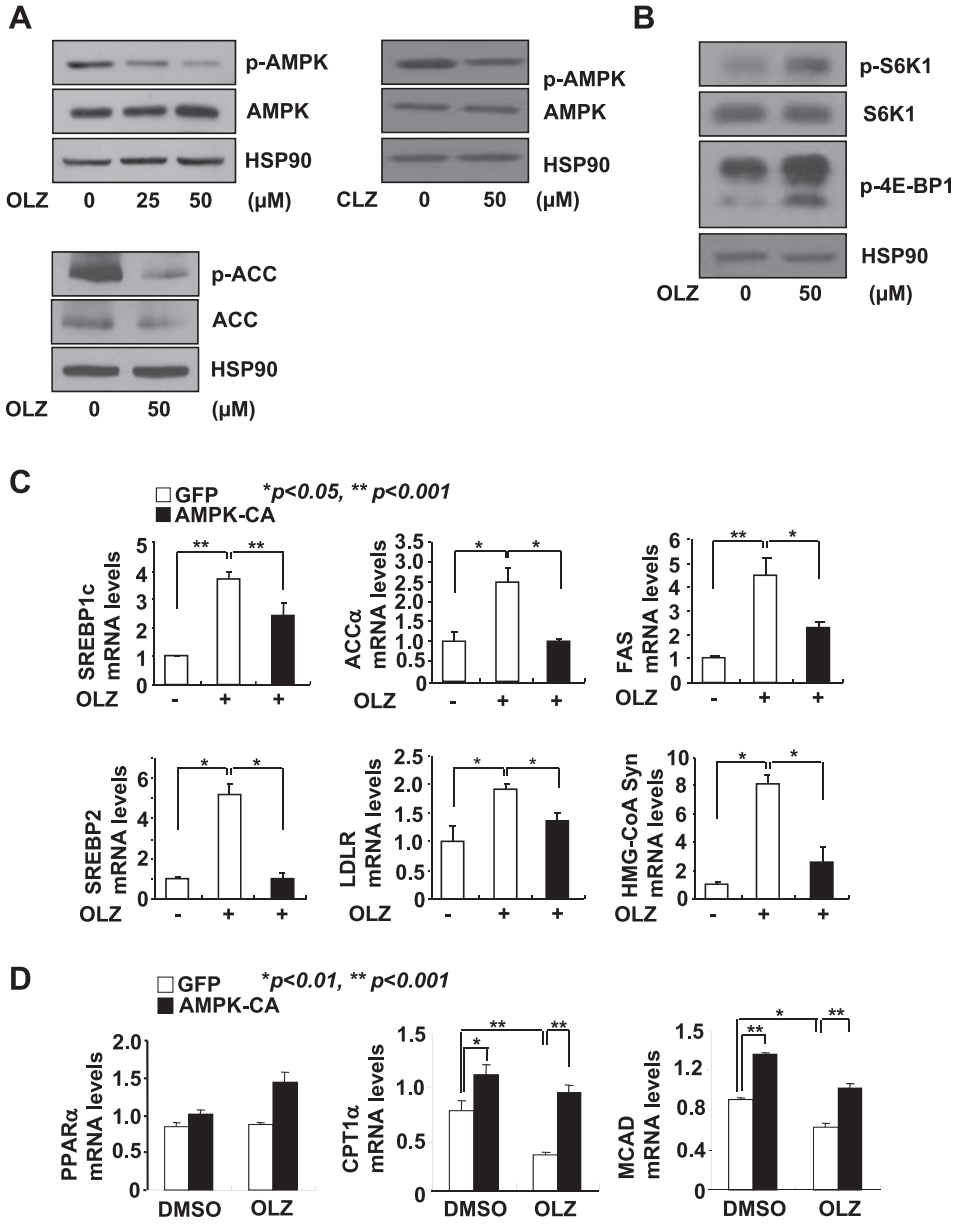

Fig. 4. AAPDs decrease AMP-activated protein kinase (AMPK) signaling to perturb lipid metabolism in primary hepatocytes. *A*: Western blot analysis showing effects of AAPDs on total and phosphorylated serine/threonine levels for AMPKα (*top*) and ACC (*bottom*). Rat primary hepatocytes were treated with DMSO, 25–50 μM OLZ, or 50 μM CLZ for 24 h. Representative data from 5 independent experiments is shown. *B*: Western blot analysis showing effects of OLZ on mammalian target of rapamycin (mTOR) activation by examining phosphorylated p70 ribosomal protein S6 kinase 1 (S6K1) and phosphorylated eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). Rat primary hepatocytes were treated with DMSO or 50 μM OLZ for 24 h. Representative data from 3 independent experiments is shown. *C* and *D*: rat primary hepatocytes were infected with Ad-GFP or adenovirus expressing constitutively active AMPK (Ad-AMPK-CA) and then treated with DMSO or 50 μM OLZ for 24 h. Quantitative PCR analysis showing effects of Ad-AMPK-CA on OLZ-induced expression of lipid synthetic genes (*C*) and fatty acid oxidation genes (PPARα, CPT Iα, and MCAD) (*D*). Data in *C* and *D* represent means ± SD.

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Mutant PPARα constructs were kindly provided by Dr. Jae-Bum Kim. Cells were treated with either DMSO or 25–50 μM OLZ for 24 h. Luciferase activity was normalized to β-galactosidase activity.

De novo lipogenesis. De novo lipogenesis activities were determined by the incorporation of [^{14}C]acetate into the lipids (16). Rat primary hepatocytes were plated in medium 199 and cultured overnight at 37°C. After the incubation period, the hepatocytes were treated with or without OLZ for 24 h in serum-free or serum-containing medium. Cells were labeled with 2 μCi [2-^{14}C]sodium acetate (American Radiolabeled Chemicals, St. Louis, MO) for 1 h before being harvested. Lipids were extracted from the cells with methanol and chloroform according to the Folch method (8). Radioactivities in the lipid fraction were counted with a liquid scintillation counter.

Measurement of metabolites. Blood glucose for basal conditions was monitored from tail vein blood using an automatic glucose monitor (OneTouch; LifeScan). Plasma and liver triglyceride levels and 3-hydroxybutyric acid were measured by colorimetric assay kits [triacylglycerol (TG), Wako; and 3-hydroxybutyric acid, BioAssay Systems]. Total liver lipids were extracted with a chloroform-methanol (2:1, vol/vol) mixture according to the Folch method (8).

Statistical analysis. Results are shown as means ± SD. Differences between two groups were assessed using two-tailed unpaired Student's *t*-test, and comparisons among more than two groups were carried out using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. A P value <0.05 was considered statistically significant and is indicated in all figures.

RESULTS

AAPDs promote lipid synthesis through regulation of SREBP transcription in primary hepatocytes. Recently, it was shown that the AAPD-dependent induction of hepatic lipogenic and cholesterogenic gene expression is mediated by SREBP proteins in cultured hepatocytes, suggesting that AAPDs might directly promote lipid synthesis in peripheral tissues (33). In line with previous reports, OLZ treatment promoted lipid accumulation and de novo lipogenesis in primary hepatocytes (Fig. 1, *A* and *B*). We were also able to observe an increase in expression of key genes in the fatty acid synthesis (SREBP-1c, ACC, FAS, and SPOT14) and cholesterol metabolism (SREBP-2, LDLR, HMG-CoA synthase, and HMG-CoA reductase) by either OLZ or CLZ in primary hepatocytes, suggesting that AAPDs are able to modulate hepatic lipid synthesis via a transcriptional mechanism (Fig. 1, *C* and *D*). To directly assess the role of SREBPs on AAPD-induced expression of hepatic lipid synthetic genes, we generated adenovirus for SREBP-DN and infected it into rat primary hepatocytes (Fig. 2*A*). AAPD-induced expression of lipogenic (ACCα and FAS) and cholesterogenic (LDLR and HMG-CoA reductase) genes was suppressed by SREBP-DN (Fig. 2*A*), suggesting that the effect of OLZ might be directly mediated by SREBPs. To further investigate the OLZ-dependent transcriptional regulation of SREBP target genes, we generated adenovirus for a luciferase reporter containing a sterol regulatory element (SRE-1) and tested it in rat primary hepatocytes (Fig. 2*B*). Indeed, AAPD-dependent induction of SRE-1 promoter activity was decreased 3.5-fold in the presence of adenovirus for dominant negative SREBP (Ad-SREBP-DN; Fig. 2*B*), showing that AAPDs regulate hepatic lipid synthesis by controlling transcriptional activity of SREBP.

AAPDs inhibit expression of fatty acid oxidation genes in primary hepatocytes. Hepatic lipid metabolism is tightly regulated by a balance between energy-requiring lipid synthetic pathway (anabolism) and energy-releasing β-oxidation pathway (catabolism). Thus, we attempted to investigate whether AAPD-dependent accumulation of hepatic lipid can also result from the decreased fatty acid oxidation. Indeed, genes involved in the fatty acid oxidation, such as PGC-1α, CPT Iα, and MCAD, were all downregulated by either OLZ or CLZ treatment in primary hepatocytes (Fig. 3*A*). To test a potential role of SREBPs on AAPD-dependent regulation of fatty acid oxidation genes, primary hepatocytes were infected with Ad-SREBP-DN in the presence of OLZ. However, unlike the case for lipogenic or cholesterogenic genes (Fig. 2*A*), infection of Ad-SREBP-DN did not restore the OLZ-dependent changes in expression of fatty acid oxidation genes, suggesting that SREBPs are not directly involved in AAPD-dependent regulation of fatty acid oxidation (Fig. 3*B*).

AAPDs target AMPK to perturb hepatic lipid homeostasis. Activation of AMPK in liver leads to both stimulation of fatty acid oxidation and inhibition of lipid and cholesterol synthesis, suggesting that AAPDs may perturb hepatic lipid metabolism by regulating AMPK activity (34, 47). Indeed, AAPD treatment reduced AMPK activity, as evidenced by reduced phos-

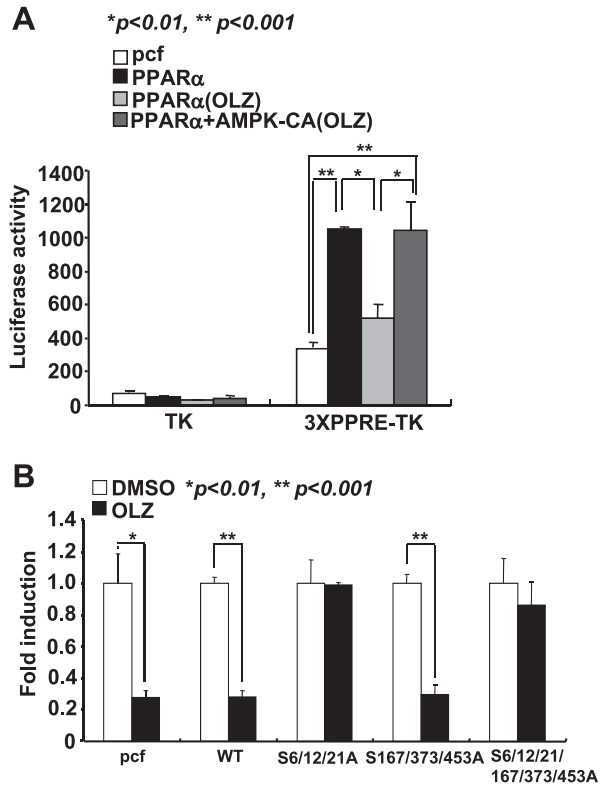

Fig. 5. AMPK restores AAPD-mediated inhibition on transcriptional activity of PPARα. *A*: transient transfection assay showing effects of Ad-AMPK-CA on OLZ-reduced transcriptional activity of PPARα. HepG2 cells were treated with DMSO or 50 μM OLZ for 24 h after cotransfection of 3X PPAR response element (PPRE)- thymidine kinase (TK) luciferase construct with pcf, PPARα wild type, or AMPK-CA. Representative data from 6 independent experiments (n = 3 for each condition) is shown. *B*: transient transfection assay showing effects of mutation of putative AMPK phosphorylation sites (S167/373/453A), known p38 MAPK phosphorylation sites (S6/12/21A), or both sites (double mutants) on PPARα. HepG2 cells were treated with DMSO or 50 μM OLZ for 24 h after cotransfection of 3X PPRE-TK luciferase construct with pcf, wild type, or mutant forms of PPARα. Representative data from 3 independent experiments (n = 3 for each condition) is shown. Data represent means ± SD.

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phorylation of AMPK at Thr[172] and ACC at Ser[79] (Fig. 4*A*). Furthermore, phosphorylation of p70 ribosomal protein S6 kinase 1 (S6K1) and 4E-BP1, downstream targets of mammalian target of rapamycin (mTOR), was also induced, indicating that AAPDs also inhibit AMPK-dependent downregulation of mTOR activity (Fig. 4*B*). To directly test whether AAPD-mediated suppression of AMPK activity is the main cause for the dysregulation of lipid homeostasis, we utilized adenovirus expressing constitutively active AMPK (Ad-AMPK-CA). Infection of Ad-AMPK-CA did not affect basal expression levels of lipogenic and cholesterogenic genes perhaps because of the minimal expression of such genes in the absence of insulin or OLZ (data not shown). However, we were able to observe that OLZ or insulin-dependent induction of SREBPs and their target gene expression were significantly reduced in the presence of AMPK-CA. (Fig. 4*C*). Similarly, infection of Ad-AMPK-CA relieved basal or OLZ-mediated repression of fatty acid oxidation genes in primary hepatocytes (Fig. 4*D*). These data confirmed that AAPDs target AMPK to disturb hepatic lipid homeostasis.

AMPK restores AAPD-mediated inhibition on transcriptional activity of PPARα. PPARα is a critical transcriptional regulator for controlling hepatic fatty acid oxidation (15, 46). AMPK has been shown to potentiate the activity of PPARα, thus influencing expression of its downstream target genes (2, 42). To further understand the mechanism of AAPD-dependent repression of AMPK on expression of fatty acid oxidation genes, we tested whether AAPDs can directly reduce PPARα transcriptional activity. As expected, OLZ decreased the ability of PPARα to transcriptionally stimulate PPRE promoter activity, whereas AMPK-CA expression restored it close to the normal level (Fig. 5*A*), suggesting that PPARα is a potential proximal target of AAPDs. To investigate the molecular mechanism by which AAPDs and AMPK regulate transcriptional activity of PPARα, we generated three PPARα expression constructs: one that carries mutations in the putative AMPK phosphorylation sites (S167/373/453A), another that carries mutations in the known p38 MAPK phosphorylation sites (S6/12/21A), and another that carries mutations in both sites (double mutants). p38 MAPK was previously shown to be

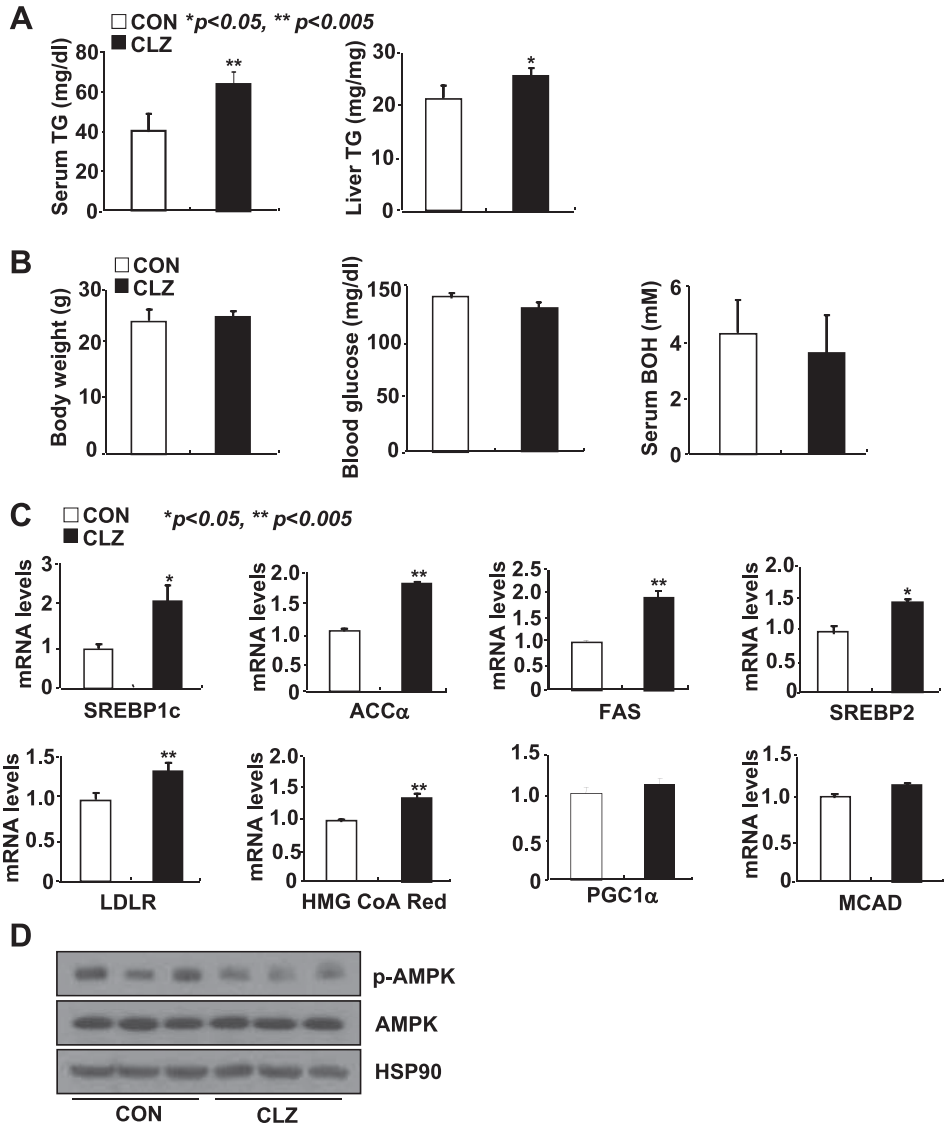

Fig. 6. AAPDs regulate lipid metabolism in vivo. Eight-week-old male C57BL/6 mice were provided with dough alone or dough containing 0.1 mg/g CLZ for 5 wk. *A*: effects of CLZ on plasma and liver triglyceride levels in mice ($n = 5$). *B*: effects of CLZ on body weight, blood glucose, and serum 3-hydroxybutyric acid (BOH) level in mice ($n = 5$). *C*: quantitative PCR analysis showing effects of CLZ on expression of lipid synthetic and fatty acid oxidation genes in mice ($n = 4$). *D*: Western blot analysis showing effects of CLZ on total and phospho-Ser/Thr protein levels of AMPKα in mice. Representative data from 3 independent experiments ($n = 4$ for each condition) is shown. Data in *A–C* represent means ± SD. CON, control; HSP90, heat shock protein 90.

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downstream of AMPK and could affect transcriptional activity of PPARα (42). As shown in Fig. 5*B*, OLZ could still reduce the transcriptional activity of S167/373/437A mutant. However, OLZ was unable to repress the transcriptional activity of either S6/12/21A mutant or double mutant, suggesting that AAPD-dependent downregulation of PPARα activity is mediated via the AMPK-p38 MAPK pathway.

AAPDs regulate hepatic lipid metabolism in vivo. Finally, to confirm the effects of AAPDs on hepatic lipid metabolism in vivo, we fed wild-type C57BL/6 mice either a control diet or CLZ-containing diet. Five-week feeding of CLZ significantly increased serum TG levels as well as hepatic TG contents (Fig. 6*A*). Interestingly, body weight was not altered by CLZ consumption, indicating that a 5-wk period may not be enough to provoke changes in body weight (Fig. 6*B*, *left*). Alternatively, a balance between physical activity and food consumption possibly hinders the detection of subtle changes in total body mass. Blood glucose levels and serum β-hydroxybutyrate tend to be lower in the CLZ-fed group than in the control group, although it did not reach the statistical significance (Fig. 6*B*, *middle* and *right*). As was the case for in vitro studies, CLZ feeding greatly induced expression of both lipogenic genes (SREBP-1c, ACCα, and FAS) and cholesterogenic genes (SREBP-2, LDLR, and HMG-CoA reductase) (Fig. 6*C*). On the other hand, no significant changes were shown in the expression levels of fatty acid oxidation genes (PGC-1α and MCAD), which was in line with subtle changes in serum ketone bodies upon CLZ feeding. CLZ feeding also slightly affected phosphorylation of hepatic AMPK (Fig. 6*D*). These data suggest that, although CLZ feeding is able to induce hepatic lipogenic program in vivo, potential secondary effects from other tissues may hinder the detection of subtle changes in lipid metabolism in liver. Collectively, we propose that AAPDs promote hepatic lipid accumulation in part via a direct action on peripheral AMPK signalings and SREBP-mediated transcriptional cascades in vivo.

DISCUSSION

The present study reports a novel role of AMPK in AAPD-induced lipid accumulation in hepatocytes. It has recently been reported that OLZ treatment could cause metabolic disorders such as dyslipidemia (5, 23). AAPDs also stimulate lipogenesis in cultured adipocytes, glioma cells, and hepatocytes, hinting that SREBP-mediated stimulation of lipogenesis in peripheral tissues could be a major mechanism for antipsychotic drug-mediated metabolic side effects (6, 32, 41). Our data show that AAPDs increase expression of SREBP-1c and its downstream target genes such as ACCα and FAS and expression of SREBP-2 and its downstream cholesterogenic genes such as HMG-CoA reductase, HMG-CoA synthase, and LDLR. These data suggest that both SREBP-1c and -2 are involved in AAPD-induced dysregulation of lipid synthesis in liver.

We confirmed the direct involvement of SREBPs on AAPD-dependent induction of lipogenic and cholesterogenic gene expression by showing that SREBP-DN blocks AAPD-mediated effects in hepatocytes. However, the exact mechanism by which AAPDs activate expression of both SREBPs was not elucidated in the current study. It is possible that AAPD-mediated induction of SREBP processing might be critical in activating its own transcription. Alternatively, AAPDs could activate other known transcription factors that activate SREBP expression.

Hepatic lipid homeostasis is tightly maintained by a balance between energy-requiring lipid synthetic pathway and energy-releasing fatty acid oxidation pathway. AMPK is shown to reduce lipid synthesis by suppressing SREBP activity and induce fatty acid oxidation in liver (9, 24, 47, 48). Therefore, the activation of AMPK may improve hyperlipidemia and the progression of the nonalcoholic fatty liver (nonalcoholic fatty liver disease) (34). We found that AAPDs were able to inhibit AMPK signalings in liver, as evidenced by the reduced phosphorylation levels of AMPKα and ACC, as well as enhanced phosphorylation of S6K1 and 4E-BP1, direct substrates for mTOR complex 1 (Fig. 4). Interestingly, recent reports suggest that mTOR signaling is responsible for the transcriptional activation of SREBP-1c and hepatic lipogenesis (22). Since AMPK was known to inhibit mTOR complex activity by direct phosphorylation of raptor (13), it is possible to postulate that AAPD-dependent activation of hepatic lipogenesis might stem from the activation of mTOR complex 1 due to the reduced AMPK activity. Further study is necessary to assess the potential contribution of AAPDs on lipid metabolism from this pathway. As mentioned previously, AMPK exerts the effects on target gene expression by modification of transcriptional machinery. It has been reported that AMPK potentiates the transcriptional activity of PPARα on downstream target genes (42). Our data show that AAPDs decreased transcriptional activity of PPARα; however, unlike the case for SREBPs, it did not affect the expression of PPARα. Rather, AAPD-mediated reduction of PPARα transcriptional activity was recovered by AMPK-CA expression (Fig. 5), suggesting that AAPDs reduced PPARα activity by regulating AMPK signaling.

Unlike the inhibitory effects of AAPDs on hepatic AMPK activity, recent reports suggest that hypothalamic AMPK activity was rather enhanced upon acute CLZ or OLZ infusion (19, 26). The differences might stem from the duration of drug treatment; whereas we performed longer-term treatment of AAPDs (24–72 h for hepatocytes and 5 wk for feeding studies), those studies focused more on the acute effect of AAPDs on hypothalamic AMPK activity. Alternatively, AAPDs may utilize tissue-specific mechanisms to affect AMPK activity.

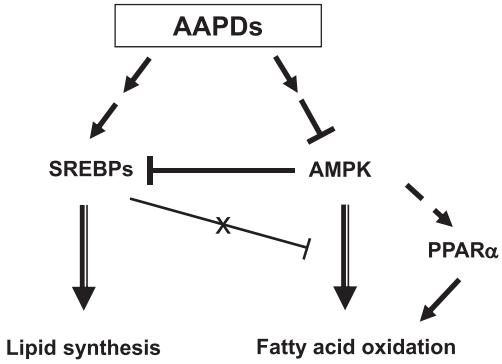

Fig. 7. A proposed model of AAPD-dependent regulation of AMPK affecting lipid synthesis and fatty acid oxidation in liver. AAPDs increase mRNA levels of SREBPs to enhance lipid synthesis, whereas they decrease PPARα-mediated fatty acid oxidation program by reducing AMPK signaling. SREBP-induced lipid synthesis might be further enhanced due to the reduction in AMPK activity.

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Although AAPDs activate AMPK through histamine H1 receptor in hypothalamus, it is unlikely that hepatic AMPK is also influenced by the same mechanism due to the lack of expression of histamine H1 receptor in liver. Interestingly, leptin is shown to influence AMPK activity differentially in a tissue-specific manner. Whereas leptin increases AMPK activity in the peripheral tissues such as the skeletal muscle, it reduces AMPK activity in the arcuate nucleus and paraventricular nucleus in the hypothalamus (27, 37), indicating that central and peripheral AMPK activities can be differentially regulated by the same stimulus. Further study is necessary to identify the potential receptor(s) for AAPDs in peripheral tissues.

The cannabinoid (CB) receptors are a class of cell membrane receptors that belong to the G protein-coupled receptor superfamily. There are currently two known subtypes termed CB_1 and CB_2. CB_1 receptors (CB1R) are expressed at high levels in the brain but are also present at much lower levels in peripheral tissues (10, 40). The activation of hepatic CB1R was shown to stimulate expression of SREBP-1c and its downstream target genes and promote de novo lipid synthesis (29). In addition, endocannabinoids and CB1R could inhibit AMPK activity and repress fatty acid oxidation in liver (17, 30). Therefore, it is possible that AAPDs might serve as a CB1R agonist and activate hepatic CB1R. In line with this idea, Weston-Green et al. (39) demonstrated that OLZ treatment significantly decreased CB receptor binding in the dorsal vagal complex. These data suggest that AAPD-induced hepatic lipid synthesis might be regulated by the modulation of CB1R. Investigations are underway to test whether hepatic CB1R are directive targets of AAPDs.

In summary, our data describe a novel relationship between AAPDs and AMPK on AAPD-mediated dysregulation of lipid metabolism in liver (Fig. 7). Thus, one could speculate that cotreatment of patients with metformin, a pharmacological drug known to activate AMPK signaling, may be useful to prevent hepatic dysregulation of lipid metabolism that seems to occur during AAPD treatment. To understand the AAPD-induced signaling pathway, it is necessary to further delineate AAPD targets in both central and peripheral tissues in in vivo models. These studies will help to further extend our understanding of how AAPDs perturb hepatic lipid metabolism to promote dyslipidemia-related syndrome and provide clues for relieving these side effects of AAPDs.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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