

p38 Mitogen-activated Protein Kinase Plays an Inhibitory Role in Hepatic Lipogenesis^{*S}

Received for publication, July 14, 2006, and in revised form, November 21, 2006. Published, JBC Papers in Press, December 17, 2006, DOI 10.1074/jbc.M606742200

Yan Xiong^{‡§}, Qu Fan Collins[‡], Jie An[¶], Edgar Lupo, Jr.[‡], Hui-Yu Liu[‡], Delong Liu[¶], Jacques Robidoux[‡], Zhenqi Liu^{**}, and Wenhong Cao^{‡††1}

From the [‡]Endocrine Biology Program, [¶]Center for Integrated Genomics, The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709, [¶]The Sarah W. Stedman Center for Nutrition and Metabolism, [§]Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha, 410078 Hunan, China, ^{**}Division of Endocrinology, Department of Internal Medicine, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, and ^{††}Division of Endocrinology, Department of Internal Medicine, Duke University, Medical Center, Durham, North Carolina 27710

Hepatic lipogenesis is the principal route to convert excess carbohydrates into fatty acids and is mainly regulated by two opposing hormones, insulin and glucagon. Although insulin stimulates hepatic lipogenesis, glucagon inhibits it. However, the mechanism by which glucagon suppresses lipogenesis remains poorly understood. In this study, we have observed that p38 mitogen-activated protein kinase plays an inhibitory role in hepatic lipogenesis. Levels of plasma triglyceride and triglyceride accumulation in the liver were both elevated when p38 activation was blocked. Expression levels of central lipogenic genes, including sterol regulatory element-binding protein-1 (*SREBP-1*), fatty acid synthase, hydroxy-3-methylglutaryl coenzyme A reductase, farnesyl pyrophosphate synthase, and cytochrome P-450-51, were decreased in liver by fasting and in primary hepatocytes by glucagon but increased by the inhibition of p38. In addition, we have shown that p38 can inhibit insulin-induced expression of key lipogenic genes in isolated hepatocytes. Our results in hepatoma cells demonstrate that p38 plays an inhibitory role in the activation of the *SREBP-1c* promoter. Finally, we have shown that transcription of the *PGC-1β* gene, a key coactivator of *SREBP-1c*, was reduced in liver by fasting and in isolated hepatocytes by glucagon. This reduction was significantly reversed by the blockade of p38. Insulin-induced expression of the *PGC-1β* gene was enhanced by the inhibition of p38 but suppressed by the activation of p38. Together, we have identified an inhibitory role for p38 in the transcription of central lipogenic genes, *SREBPs*, and *PGC-1β* and hepatic lipogenesis.

Hepatic lipogenesis is essential for maintaining energy balance (1). Disorders of hepatic lipogenesis may lead to fatty liver, dyslipidemia, type II diabetes mellitus, and complications such as atherosclerosis (2). Lipogenesis in liver includes *de novo* syn-

thesis of fatty acids and cholesterol. As a major site for synthesis of fatty acids, the liver converts excess carbohydrates into fat storage in the fed state. Fatty acids synthesized in the liver are converted into triglycerides and secreted as very low density lipoproteins, which transport fatty acids to the storage sites in adipocytes. Cholesterol synthesized in the liver are also transported to other tissues via very low density lipoproteins as essential building materials for steroid hormones and cellular membranes. However, excess production of fatty acids and cholesterol from the liver may contribute to a variety of lipid disorders. The lipogenic process in the liver is primarily regulated by central lipogenic transcription factors, sterol regulatory element-binding proteins (*SREBPs*)² (reviewed in Refs. 3 and 4).

The *SREBPs* are basic helix-loop-helix-leucine zipper-containing transcription factors (5). Among three known *SREBPs*, *SREBP-1a* and *-1c* are encoded by the same gene; *SREBP-1c* lacks the N-terminal exon compared with *SREBP-1a* (6). *SREBP-2* is encoded by a separate gene (7). Although *SREBPs* share similar lipogenic function, *SREBP-1c* is primarily involved in fatty acid synthesis, whereas *SREBP-2* is mainly involved in cholesterol synthesis (8–10). In contrast, *SREBP-1a* is a potent activator of both fatty acid and cholesterol syntheses (9, 11, 12). *SREBP-1c* and *-2* are predominant isoforms in the liver and major regulators of hepatic lipogenesis (13). *SREBPs* regulate lipogenesis by stimulating expression of their target genes, such as liver pyruvate kinase, acetyl CoA carboxylase, fatty acid synthase (*FAS*), *Spot14*, diacylglycerol acyltransferase, and glycerol-3-phosphate acyltransferase (reviewed in Ref. 4). The function of *SREBPs* is primarily regulated through protein cleavage and gene expression.

The “inactive” precursor form of *SREBPs* are integral membrane proteins of endoplasmic reticulum. Upon sterol deprivation, they are cleaved through two sequential steps to release the N terminus, which is translocated into the nucleus to activate the transcription of target genes (6, 14, 15). Transcription of *SREBP* genes in the liver is principally regulated by insulin and glucagon (16–18). Although the mechanism of *SREBP* gene transcription by these hormones has been intensively

^{*} This work was supported by the Investigator Development Fund from the The Hamner Institutes for Health Sciences (to W. C.) and American Heart Association Grant SDG-0530244N (to W. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

^S The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Figs. 1 and 2.

¹ To whom correspondence should be addressed: The Hamner Institutes for Health Sciences, Six Davis Dr., P.O. Box 12137, Research Triangle Park, NC 27709. Tel.: 919-558-1396; E-mail: wcao@thehamner.org.

² The abbreviations and trivial names used are: *SREBP*, sterol regulatory element-binding protein; TG, triglyceride; *FAS*, fatty acid synthase; *FPS*, farnesyl pyrophosphate synthase; SB, SB203580; RT, reverse transcription; HFD, high fat diet; siRNA, small interfering RNA; dn, dominant negative.

studied, there are still significant gaps in our understanding. Recently, peroxisome proliferator-activated receptor γ co-activator-1 β (PGC-1 β) has been shown to interact with SREBP-1c as a coactivator in regulating the transcription of lipogenic genes (19). However, the signaling pathway from hormones to the SREBP promoters, such as the identity of specific kinases, remains largely unknown.

p38 mitogen-activated protein kinase (p38) is one member of the mitogen-activated protein kinase superfamily. It is a cellular sensor of many stresses caused by various stimuli (reviewed in Refs. 20–22). Essentially, any significant change in extracellular environment can activate p38 and set in motion certain protective mechanisms, such as activation and production of heat shock proteins, immune responses, and apoptosis. We and others have recently shown that p38 plays an important role in the control of energy balance in brown adipocytes, muscle cells, and hepatocytes (23–28). In this study, we investigated the role of p38 in the control of hepatic lipogenesis. Our results show that p38 is activated in liver by fasting and in isolated hepatocytes by glucagon. Suppression of p38 in liver or isolated hepatocytes led to elevated expression of lipogenic genes and increased triglyceride levels in both plasma and liver. Together our results support a critical role for p38 in the regulation of hepatic lipogenesis.

MATERIALS AND METHODS

Chemicals, Antibodies, and Plasmids—SB203580 (SB) was from Calbiochem. Glucagon and Percoll were from Sigma. Antibodies against p38 and phosphorylated p38 were from Cell Signaling Technology. Antibodies against SREBP-1 were from Santa Cruz Biotechnology. The SREBP-1c promoter construct (pBP1c, 1.3 kb) was a kind gift from Drs. Michael S. Brown and Joseph L. Goldstein (29). The constructs for dominant-negative p38 α (p38(AF)) and MKK6E were kindly provided by Dr. Jiahua Han.

Animal Experiments—To examine the role of p38 in lipid metabolism, C57BL/6 mice (10 mice/group) were fed with normal (standard) chow diet or high fat diet (Research Diets catalog number D12330: 58.0 kcal% fat, 16.0 kcal% protein, and 26 kcal% carbohydrate) as noted. Six weeks later, some mice were treated with SB (30 mg/kg body weight/day through gastric gavages) or the vehicle solution for another 2 weeks as noted. SB and vehicle were administered one dose/day. At completion of these treatments, blood samples were collected for measurements of lipids, and livers were harvested for quantification of triglyceride (TG) and analyses of target proteins and mRNAs.

To further evaluate the role of p38 in expressions of lipogenic genes, C57BL/6 mice (6–8 weeks old) were fasted for 24 h in the presence or absence of SB (12.4 mg/kg body weight via immunoprecipitation) as noted. SB was administered as previously described (27). The fed mice were used as a control. Livers were collected for the measurements of lipogenic genes.

Preparation, Transfection, and Viral Infection of Primary Hepatocytes—Primary hepatocytes from C57BL/6 mice fed with normal chow diet at the regular schedule were prepared as previously described (30). Briefly, under anesthesia with pentobarbital (immunoprecipitation, 30 mg/kg body weight), livers were perfused with Hanks' balanced salt solution (Invitrogen)

at 5 ml/min for 8 min followed by continuous perfusion with serum-free Williams' medium containing collagenase (Worthington, type II, 130 units/ml) (Invitrogen), HEPES (10 mM), and NaOH (0.004 N) at 5 ml/min for 12 min. Hepatocytes were harvested and purified with Percoll as described previously (31). The viability of hepatocytes was examined with trypan blue exclusion. Cells with viability >95% were used. Hepatocytes were inoculated into collagen-coated 6-well plates (5×10^5 /well) in Williams' medium. Cells were incubated overnight before any experimentation. MKK6E and p38-AF-FLAG (dominant negative p38 α) were introduced into primary hepatocytes with Lipofectamine 2000 according to the manufacturer's manuals (Invitrogen). For adenoviral infection, 50 active viral particles/cell in 1 ml of Williams' medium with 2% fetal bovine serum were used to incubate with cells for 6 h followed by incubation with fresh medium containing 10% fetal bovine serum (32). At 36 h post-infection, levels of p38 and expression of genes in hepatocytes were detected by immunoblottings with appropriate antibodies and TaqMan real-time reverse transcription (RT)-PCR, respectively.

Culture and Transfection of Hepatoma Cell Line—The Hepa1c1c7 mouse hepatoma cell line was purchased from the American Type Culture Collection (Manassas, VA) and cultured with minimal essential α medium in the presence of 10% fetal bovine serum and antibiotics. Transient transfection of these cells was performed using Lipofectamine 2000 (Invitrogen) according to instructions from the manufacturer.

Immunoblotting—Tissue or whole-cell lysates were prepared by homogenization and sonication followed by the addition of 2 \times Laemmli sample buffer. Aliquots (5–10 μ g protein/well) were resolved with mini-Tris-glycine gels (4–20%) (Invitrogen) and transferred to nitrocellulose membranes. Levels of p38 and SREBP-1 were detected with a 1:1000 dilution of each specific antiserum (catalog numbers 9211S and 9212 from Cell Signaling Technology and catalog number sc-8984 from Santa Cruz Biotechnology) followed by a 1:10,000 dilution of goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase (RPN5783, Amersham Biosciences). Fluorescent bands were visualized with a Typhoon phosphorimaging device (Molecular Dynamics).

RNA Isolation and TaqMan Real-time RT-PCR—Total RNAs from liver were prepared by using RNA purification kits from Qiagen. Real-time RT-PCR TaqMan probes and reaction agents were purchased from Applied Biosystems. Reactions were performed according to manuals from the manufacturer. All results were normalized to levels of the GAPDH gene. Catalog numbers for the probes are: SREBP-1 (Mm00550338_m1), SREBP-2 (Mm01306293_m1), FAS (Mm00662319_m1), PGC-1 β (Mm00504720_m1), HMG CoA reductase (Mm01282491-g1), farnesyl pyrophosphate synthase (FPS) (Mm00830315_g1), and CYP51 (Mm0049968_m1).

Measurement of SREBP-1c Promoter Activity—The SREBP-1c promoter was introduced into Hepa1c1c7 hepatoma cells together with the expression vector for β -galactosidase via transient transfection (24) and stimulated as noted. SREBP-1c promoter activity was measured by luciferase assays and normalized to the internal control of transfection, β -galactosidase.

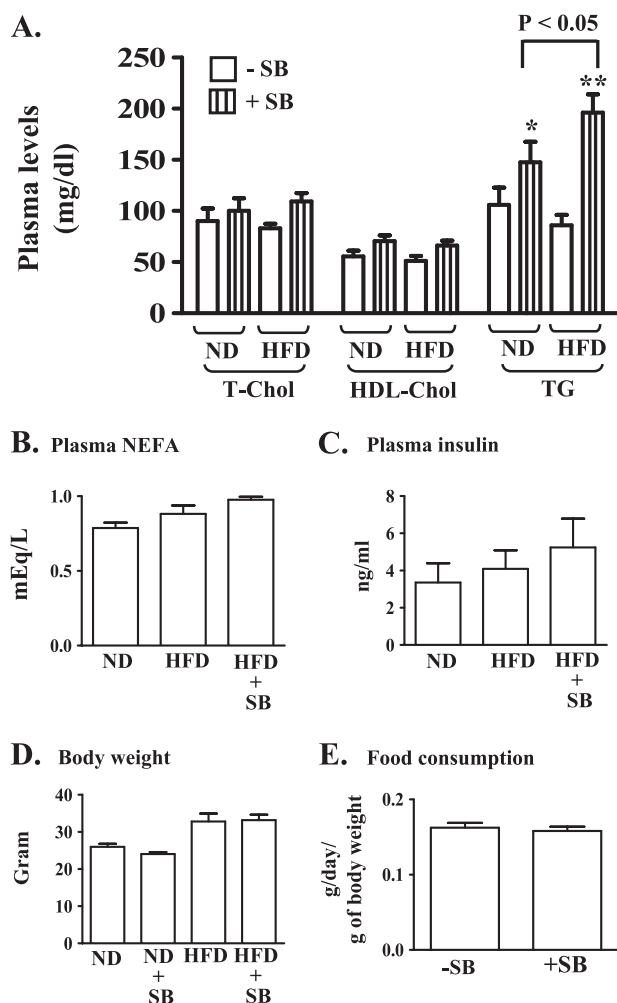


FIGURE 1. The inhibition of p38 elevates levels of plasma triglyceride. A, mice were fed with normal chow diet (ND) or high fat diet (HFD) for 8 weeks as detailed under "Materials and Methods." Some of the mice were treated with SB during the last 2 weeks as noted. Plasma levels of total cholesterol (T-Chol), high density lipoprotein (HDL), and triglycerides (TG) were measured. Results shown represent means \pm S.D. of two independent experiments, each with 5 mice/group. *, $p < 0.05$ compared with -SB; **, $p < 0.01$ versus -SB. Plasma levels of non-esterified fatty acids (NEFA) (B) and insulin (C) from these animals were measured. Body weight (D) and food intake (E) were quantified.

Measurements of mRNA Degradation—Transcription of lipogenic genes in hepatocytes were activated by insulin and dexamethasone for 20 h and subsequently blocked by treatment with amanitin as described previously (33). Cells were then treated with either SB or vehicle solution as noted. Levels of representatives of lipogenic genes (SREBP-1c and PGC-1 β) were quantified with TaqMan real-time RT-PCR.

Measurements of Liver Triglyceride Content—Livers were homogenized in a buffer containing 18 mM Tris (pH 7.4), 300 mM D-mannitol, 50 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride. Triglycerides in the lysates were extracted using a chloroform/methanol protocol as previously described (34). Levels of triglycerides were quantified by using triglyceride reagents from Sigma (catalog number T2449-10ML) and normalized to protein concentration. Lipid droplets in liver slides were visualized with Oil Red O staining.

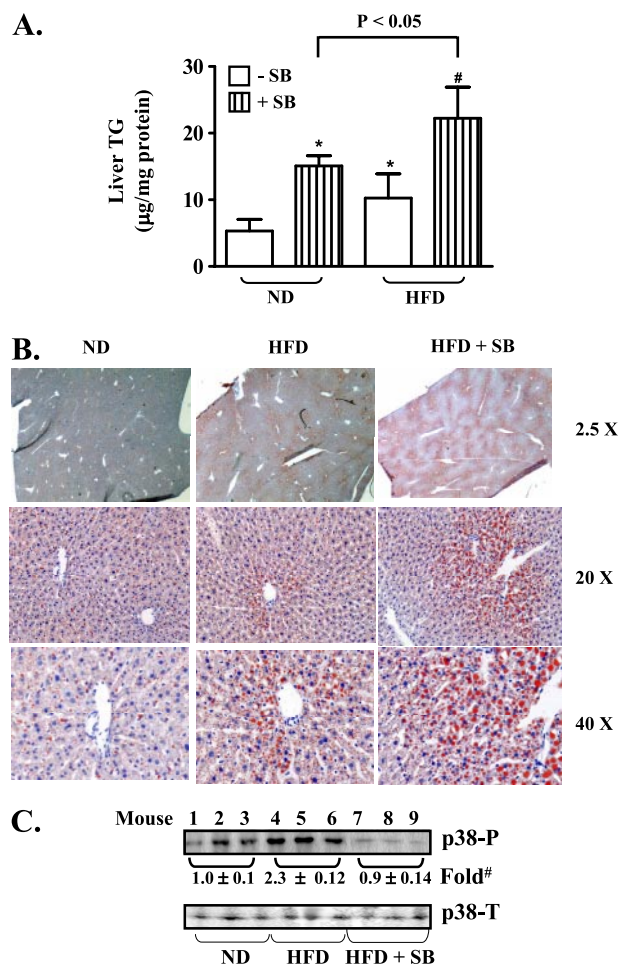


FIGURE 2. The inhibition of p38 enhances fat accumulation in liver. Mice were treated in the same way as described in Fig. 1. A, levels of TG content in liver were quantified with a standard method. *, $p < 0.05$ compared with ND with no SB treatment. #, $p < 0.01$ compared with -SB under HFD. B, lipid content in liver tissue was visualized with Oil Red O staining. C, levels of p38 phosphorylation in liver were detected by immunoblotting with antibodies against total or phospho-p38. Results shown represent means \pm S.D. of two independent experiments. #, $p < 0.05$ comparing HFD to either ND or HFD + SB.

RESULTS

Inhibition of p38 Leads to Elevation of Plasma Lipids and Fat Accumulation in Liver—During our study on the stimulatory role of p38 in hepatic gluconeogenesis (27), we unexpectedly observed that p38 might inhibit hepatic lipogenesis. To fully characterize this observation, we examined the effect of p38 inhibition on levels of plasma lipids and liver TG content in mice. As shown in Fig. 1A, although plasma levels of total cholesterol (T-Chol), high density lipoprotein cholesterol (HDL-Chol), and TGs were not significantly changed after high fat diet (HFD) for 8 weeks, hepatic TG content was significantly increased by HFD ($p < 0.05$) (Fig. 2A). Levels of TG in both plasma and liver were significantly increased by the inhibition of p38 in the mice under either normal chow or high fat diet. (Figs. 1A and 2A). Both the number and size of the lipid droplets in the liver were increased by HFD, and these increases were further significantly aggravated by the inhibition of p38 (Fig. 2B). Levels of phosphorylated p38 in the liver were also enhanced by HFD, but this effect was blocked by SB (Fig. 2C). It

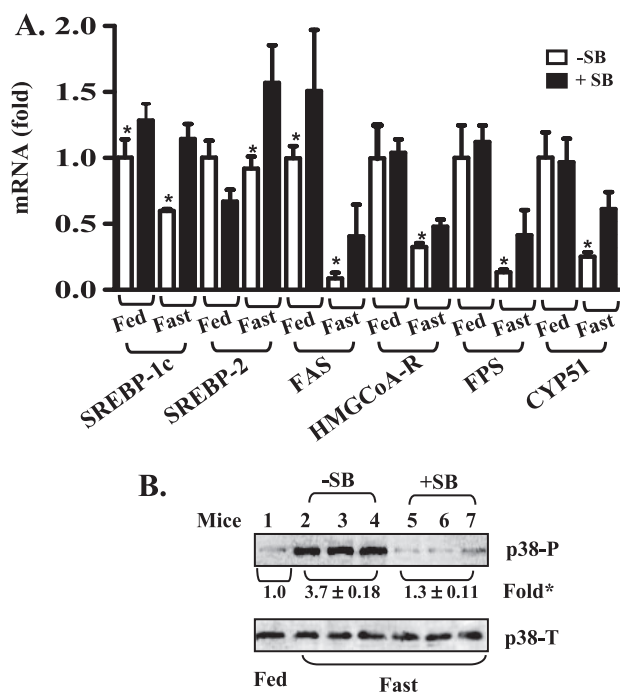


FIGURE 3. p38 plays an inhibitory role in expression of key lipogenic genes in liver. Mice were fasted for 24 h in the presence or absence of p38 inhibitor SB. Fed mice were also treated with SB as noted. *A*, transcripts of *SREBP-1c*, *SREBP-2*, *FAS*, *HMG CoA-R*, *FPS*, and *CYP51* genes in liver were quantified with TaqMan real-time RT-PCR. Results shown represent means \pm S.D. of two independent experiments, each with 4 mice/group. *, $p < 0.05$ compared with +SB. *B*, levels of p38 phosphorylation in liver were detected by immunoblotting with antibodies against either total or phospho-p38. *, $p < 0.05$ comparing fasting without SB to fasting with SB.

was noted that treatment of animals with SB did not significantly influence either levels of plasma non-esterified fatty acids and insulin or body weight and food consumption (Fig. 1, *B–E*). Together, these results show that the blockade of p38 can elevate TG levels in both plasma and liver, suggesting an inhibitory role for p38 in hepatic lipogenesis.

The Blockade of p38 Can Increase Transcript Levels of Lipogenic Genes in Fasted Liver—To investigate the possible role of p38 in suppression of hepatic lipogenesis, we chose to use fasting mice as models, because it is well known that hepatic lipogenesis is normally inhibited by fasting and p38 is activated in the liver during fasting (27). As expected (Fig. 3*A*), levels of lipogenic gene transcripts, including *SREBP-1c*, *FAS*, hydroxy-3-methylglutaryl coenzyme A reductase, *FPS*, and *CYP51* were decreased by fasting, and these decreases were significantly, although not completely, reversed by the inhibition of p38. *SREBP-2* transcripts were not reduced by fasting but were significantly elevated upon the blockade of p38. Levels of both *SREBP-1c* and *FAS* transcripts in fed mice were also increased by SB. Phosphorylation of p38 in the liver was increased by fasting and inhibited by SB (Fig. 3*B*) as expected (27). These results indicate that activation of p38 in liver is related to the reduction of transcript levels of central lipogenic genes, *SREBP-1c*, and its downstream target genes.

p38 Plays a Suppressive Role in Transcription of Lipogenic Genes in Primary Hepatocytes—Lipogenesis is inhibited during fasting to protect the substrate supply for glucose production in liver (16). The predominant suppressor of lipogenesis is glu-

cagon, which is also the major promoter of hepatic glucose production and is always significantly elevated in the blood during fasting (35). Glucagon is a cAMP-producing hormone that can activate p38 in primary hepatocytes (27, 36). To determine whether p38 mediates glucagon suppression of hepatic lipogenesis, levels of key lipogenic gene transcripts and phosphorylation of p38 in primary hepatocytes were examined in the presence or absence of p38 blockade. As predicted, levels of *SREBP-1c*, *SREBP-2*, *FAS*, *HMG CoA-R*, and *FPS* transcripts were inhibited by glucagon (Fig. 4*A*). This inhibition was significantly reversed by the blockade of p38 with either SB or overexpression of dominant negative p38 α (dn-p38 α). To determine the role of p38 in insulin-induced transcription of key lipogenic genes, primary hepatocytes were stimulated by insulin in the presence of a p38 inhibitor or an activator. As shown in Fig. 4*B*, levels of key lipogenic genes *SREBP-1c* and *FAS* were elevated by insulin as expected, and this elevation was further enhanced by the inhibition of p38 with SB but prevented by the activation of p38 with MKK6E. Furthermore, levels of the *SREBP-1* protein (membrane-bound) were significantly increased by all p38 inhibitors, including SB, dn-p38 α , and small interfering RNA against p38 α (siRNA-p38 α) but decreased by glucagon (Fig. 4*C*). The nuclear form of *SREBP-1* was also slightly elevated by the inhibition of p38. The scrambled siRNA had no effect. (Note: cells were not deprived of cholesterol in these experiments.) Phosphorylation of p38 in isolated hepatocytes was also stimulated by glucagon and blocked by either SB or siRNA-p38 α (Fig. 4*D*). Our results indicated that the inhibition of p38 did not affect the stability of *SREBP-1c* mRNA (supplemental Fig. 1). Together, these results support the notion that p38 is an inhibitor of lipogenic gene expression in isolated hepatocytes.

p38 Is an Inhibitor of the *SREBP-1c* Promoter—To further define the role of p38 in hepatic lipogenesis, we examined the role of p38 in activation of the *SREBP-1c* promoter, which was introduced into Hepa1c1c7 hepatoma cells via transient transfection. Because insulin is known to stimulate transcription of the *SREBP-1c* gene (17, 37–39), activity of the *SREBP-1c* promoter was stimulated with insulin in the presence or absence of a p38 inhibitor. As shown in Fig. 5*A*, the *SREBP-1c* promoter was stimulated by insulin as expected, and this stimulation was further enhanced by the inhibition of p38. Similarly, the *SREBP-1c* promoter was suppressed by glucagon as expected, and the suppression was significantly reversed by the inhibition of p38 with either SB or siRNA (Fig. 5*B*). Together, these results further support an inhibitory role for p38 in the transcription of the central lipogenic gene, *SREBP-1c*.

p38 Can Inhibit Transcription of the *PGC-1 β* Gene in Both Liver and Isolated Hepatocytes—PGC-1 β was recently shown to be a critical coactivator of lipogenic gene expression through its interaction with *SREBP-1c* (19). To examine whether transcription of the *PGC-1 β* gene is a target for p38 in the suppression of hepatic lipogenesis, we examined levels of *PGC-1 β* transcripts in livers from fed or fasted mice with or without treatment with SB. As shown in Fig. 6*A*, levels of *PGC-1 β* transcripts in liver were decreased by fasting; this decline was significantly, although not completely, reversed by the blockade of p38. To more directly study the effect of p38 on levels of

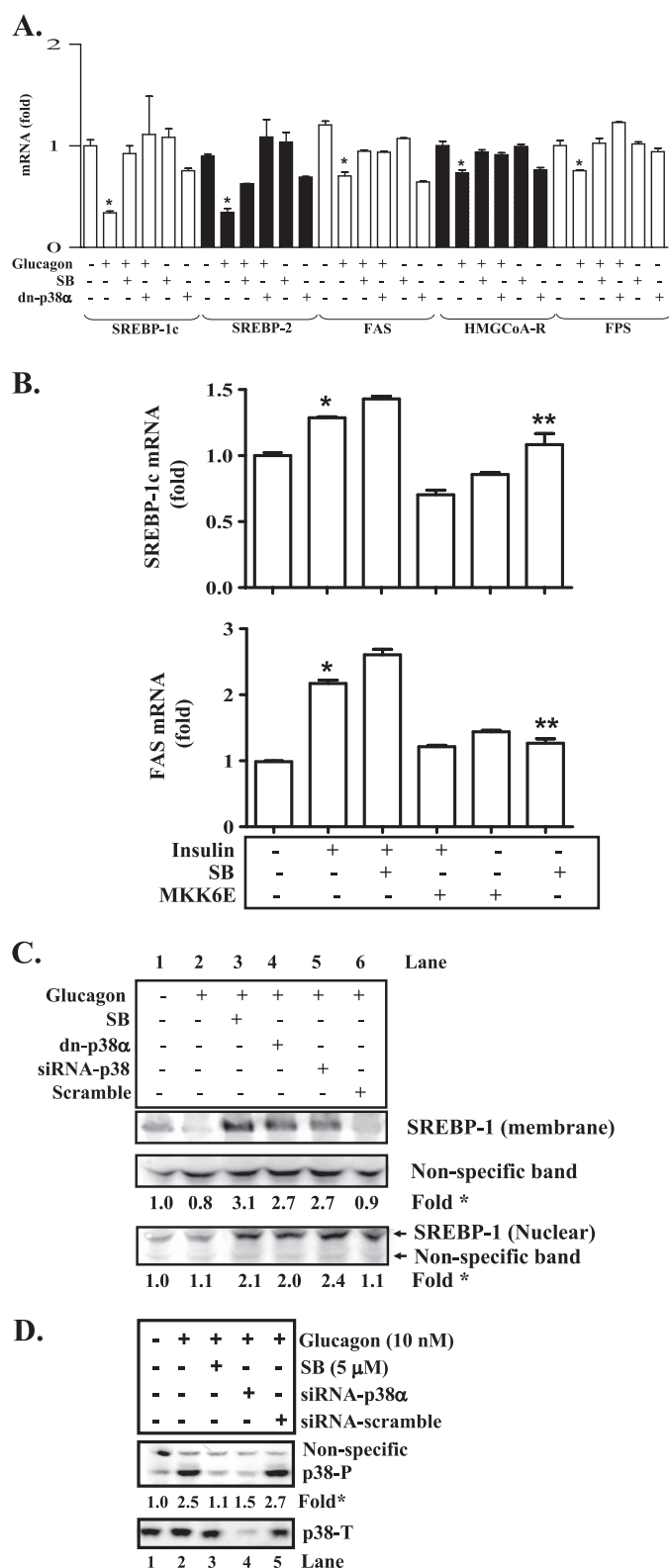


FIGURE 4. p38 plays an inhibitory role in transcription of lipogenic genes in primary hepatocytes. Primary hepatocytes were isolated and cultured as detailed under "Materials and Methods." **A**, primary hepatocytes were stimulated with glucagon (50 nM, 16 h) in the presence or absence of p38 inhibitor SB (5 μM) or dominant negative p38α (dn-p38α). Transcripts of *SREBP-1c*, *SREBP-2*, *FAS*, *HMGCoA-R*, and *FPS* genes were quantified with TaqMan real-time RT-PCR. Results shown represent means \pm S.D. of three experiments. *, $p < 0.05$ compared with all other treatments. **B**, primary hepatocytes were treated with insulin (100 nM) in the presence or absence of SB

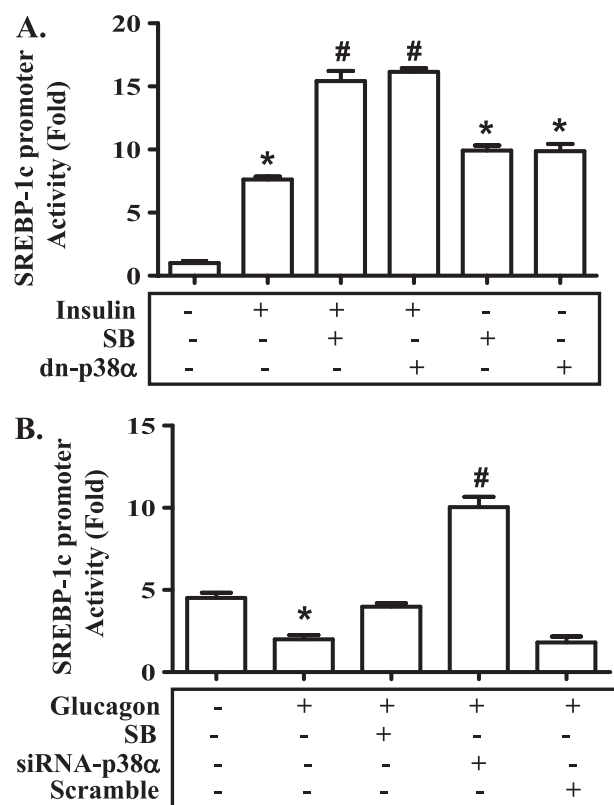


FIGURE 5. p38 inhibits activation of the SREBP-1c promoter. The SREBP-1c promoter was introduced into Hepa1c1c7 cells via transient transfection. Cells were then treated for 16 h with either insulin (50 nM) (**A**) or glucagon (50 nM) (**B**) in the presence of p38 inhibitors (5 μM SB, dominant negative-p38α, or siRNA against p38α) as noted. Promoter activities were subsequently quantified with luciferase assays and normalized to β-galactosidase activity (internal control). Results shown represent means \pm S.D. of two independent experiments, each in triplicate. *, $p < 0.05$ compared with the basal level or to inhibition of p38; #, $p < 0.05$ versus all other treatments.

PGC-1β transcripts, primary hepatocytes were treated with glucagon in the presence or absence of p38 inhibition. As shown in Fig. 6B, levels of PGC-1β transcripts were reduced by glucagon, but the reduction was completely prevented by the blockade of p38 with either SB or dn-p38α. Similarly, insulin-induced expression of the *PGC-1β* gene was also enhanced by the inhibition of p38 but suppressed by the activation of p38 (Fig. 6C). To determine the possible role of p38 in the degradation of PGC-1β mRNA, the transcription of the *PGC-1β* gene was first stimulated by insulin and dexamethasone and then blocked by amanitin followed by treatment with SB. As shown in Fig. 6D, treatment with SB did not significantly influence levels of PGC-1β transcripts during a 24-h degradation course.

or MKK6E as noted followed by measurements of SREBP-1c and FAS transcripts by TaqMan real-time RT-PCR. *, $p < 0.05$ versus all other treatments. **, $p < 0.05$ versus basal. **C**, primary hepatocytes were stimulated by glucagon (50 nM, 16 h) in the presence or absence of SB, dn-p38α, or siRNA against p38α (siRNA-p38α). The scrambled siRNA was used as a control. Protein levels of membrane-bound and nuclear SREBP-1 in these cells were assessed by immunoblotting with antibodies against SREBP-1. *, $p < 0.05$ comparing lanes 3, 4, or 5 to lanes 1, 2, or 6. **D**, primary hepatocytes were treated with glucagon (50 nM, 30 min) in the presence or absence of SB or siRNA-p38α encoded by adenoviruses as detailed under "Materials and Methods." Levels of total and phospho-p38 were also detected by immunoblotting. Results shown represent means \pm S.D. of three experiments. *, $p < 0.05$ comparing phospho-p38 in either lanes 2 or 5 to lanes 1, 3, or 4.

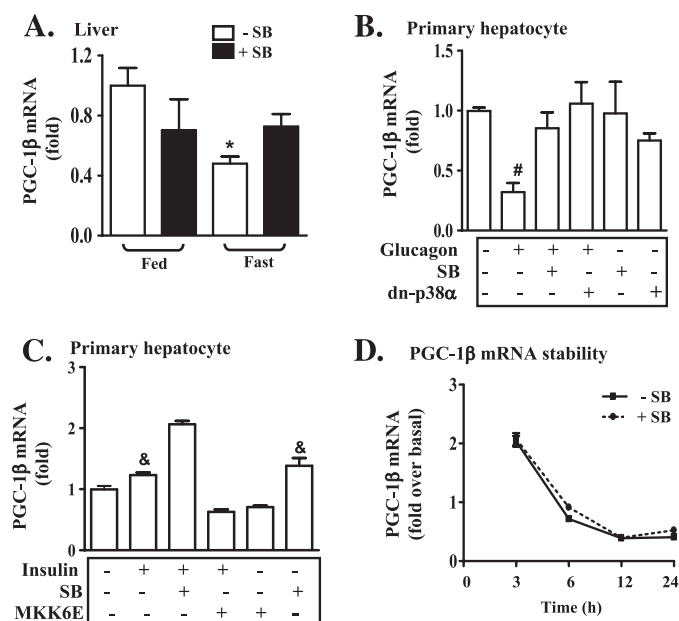


FIGURE 6. p38 suppresses expression of the *PGC-1β* gene in liver and primary hepatocytes. *A*, levels of *PGC-1β* transcripts in livers from the mice described in Fig. 3 were detected by TaqMan real-time RT-PCR. *, $p < 0.05$ compared with fed or fast + SB. *B*, isolated primary hepatocytes were treated with glucagon (50 nM, 16 h) in the presence or absence of p38 inhibition with either SB (5 μ M) or dn-p38 α . Transcripts of the *PGC-1β* gene were quantified by TaqMan real-time RT-PCR. Results shown represent means \pm S.D. of three independent experiments. #, $p < 0.05$ compared with all other treatments. *C*, isolated primary hepatocytes were treated with insulin (100 nM, 16 h) in the presence or absence of either the p38 inhibitor SB (5 μ M) or overexpression of p38 activator MKK6E. Transcripts of the *PGC-1β* gene were quantified by TaqMan real-time RT-PCR. Results shown represent means \pm S.D. of two independent experiments. &, $p < 0.05$ compared with all other treatments. *D*, hepatocytes were sequentially treated with insulin (100 nM) and dexamethasone (10 nM) for 20 h and α -amanitin (15 μ M) for 3 h followed by treatment with SB (5 μ M) or vehicle solution for the time indicated. Levels of *PGC-1β* transcripts were then quantified by TaqMan real-time PCR. Results represent mean \pm S.D. of two independent experiments, each in triplicate.

Together, these results suggest that p38 suppresses transcription of the *PGC-1β* gene.

DISCUSSION

Much is known about how hepatic lipogenesis is stimulated by metabolically important hormones such as insulin, whereas little is known about the mechanism by which hepatic lipogenesis is suppressed. It is generally accepted that glucagon is the major inhibitor of lipogenesis during fasting due to its activation of the cAMP/protein kinase A pathway (see Ref. 40 for review). Nevertheless, the mechanism(s) beyond protein kinase A have not been well defined. In this study, we have identified a critical role for p38 in the regulation of hepatic lipogenesis. Specifically, the blockade of p38 leads to hypertriglyceridemia and fatty liver.

Many factors may contribute to the development of the hypertriglyceridemia and fatty liver observed in this study. Because p38 has been shown to play a stimulatory role in glucose uptake in skeletal muscles and adipocytes through expression of *Glut4* (41–49), the blockade of p38 may reduce glucose uptake in these tissues, resulting in excess glucose supply. The excess glucose is then converted into fatty acids and contributes to the development of hypertriglyceridemia and fatty liver. The blockade of p38 may also cause fat accumulation by reducing

oxidation of fatty acids, as p38 has been shown to promote activation and expression of *PGC-1α* (24, 27, 50), which is a critical player in skeletal muscles and hepatocytes in promoting fatty acid oxidation (51). We have previously reported that the blockade of p38 can prevent activation of cAMP response element-binding protein in both liver and isolated hepatocytes (27, 28). cAMP response element-binding protein has been previously shown to inhibit hepatic lipogenesis through stimulating expression of the Hairy Enhancer of Split-1 (*HES-1*) gene and consequently suppressing expression of the peroxisome proliferator-activated receptor γ (*PPARγ*) gene (52). Therefore, the inhibition of p38 seems to contribute to fat accumulation in the liver by suppressing activation of cAMP response element-binding protein. However, our results show that expression of the *HES-1* gene was not significantly affected by the blockade of p38, whereas expression of the *PPARγ* gene was decreased instead of increased (supplemental Fig. 2). Thus, it is unlikely that blockade of cAMP response element-binding protein-mediated suppression of hepatic lipogenesis contributes to the fatty liver seen in this study. Additionally, the blockade of triglyceride export from hepatocytes may also cause fatty liver. Our results show that the blockade of p38 indeed decreases mRNAs of the microsomal triglyceride transport protein gene in liver (supplemental Fig. 2). Finally, our results clearly show that p38 plays an inhibitory role in the transcription of key lipogenic genes in both liver and isolated hepatocytes. Therefore, it is obvious that multiple factors contributed to the hypertriglyceridemia and fatty liver caused by the blockade of p38.

Although p38, along with c-Jun NH₂-terminal kinase (the so-called stress-activated kinases), was originally linked to stress and cell death (20–22), it has since been recognized to be involved in a variety of other cellular functions such as development and differentiation (see Ref. 22 for review). Recently, we and others have observed that p38 plays an important role in energy balance through its involvement in the transcription of several genes in brown adipocytes, muscle cells, and hepatocytes, which regulate oxidative metabolism, thermogenesis, and glucose production (23–25, 27, 28, 50, 53). It has been previously shown (27, 36) in hepatocytes that p38 could be activated by cAMP-generating hormones, including glucagon. Because glucagon is the major hormone elevated in blood during fasting and has previously been shown in hepatocytes to be able to suppress expression of the central lipogenic gene SREBP-1c (17), we postulated that p38 might mediate glucagon suppression of hepatic lipogenesis. Our results in this study strongly support this hypothesis, as levels of plasma TG and hepatic TG content, along with expression of key lipogenic genes including SREBP-1c, were significantly enhanced in liver when p38 was inhibited in animals. In addition, our results from isolated hepatocytes and promoter analysis also show that p38 inhibits the transcription of the central lipogenic gene SREBP-1c. The exact mechanism by which p38 regulates lipogenesis in the liver is still not established yet.

PGC-1β appears to be an important mediator of p38 in the regulation of hepatic lipogenesis, as our results show that p38 can inhibit expression of the *PGC-1β* gene, which was recently shown to be a necessary coactivator of SREBP-1c in the transactivation of lipogenic genes, including the SREBP-1c gene

itself (19). The promoter of the SREBP-1c gene contains both the SREBP response element and the liver X receptor response element (37, 54). Transcription of the SREBP-1c gene can be auto-stimulated by SREBP proteins through the SREBP response element (55–57) or stimulated by the LXR agonists through the liver X receptor response element (37, 54). Because PGC-1 β possesses the domains for mediating its interactions with both SREBP and LXR (19), it is possible that PGC-1 β regulates the transcription of SREBP-1c and other SRE- and liver X receptor response element-containing genes through assembling the SREBP and LXR complexes, and p38 regulates transcription of key lipogenic genes (*SREBP-1c*) through expression of the *PGC-1 β* gene. It is noteworthy that Lin *et al.* (58) show an increased expression of the *PGC-1 β* gene in liver by fasting, and we here show an opposite result, *i.e.* expression of the *PGC-1 β* gene is decreased by fasting. It is currently unclear what caused this discrepancy. However, our computational analysis shows that, unlike the *PGC-1 α* promoter, which possesses a consensus cAMP response element and is expected to be activated by fasting or glucagon (59, 60), the *PGC-1 β* promoter does not contain a cAMP response element. Therefore, the *PGC-1 β* promoter is not necessarily activated by fasting or glucagon.

In summary, we have identified p38 as an important modulator of hepatic lipogenesis. Because inhibition of lipogenesis is necessary to protect the substrate supply for hepatic gluconeogenesis during fasting, this role of p38 may be pivotal for maintaining plasma glucose levels. In addition, the inhibitory role of p38 may also be important to limiting lipid synthesis in liver when excess calories, particularly fats, are ingested. The exact mechanism by which p38 inhibits transcription of the central lipogenic genes, such as *SREBP-1c* and *PGC-1 β* , requires further investigation, which may yield new targets for manipulating hepatic lipogenesis for the treatment of lipid disorders.

Acknowledgments—We thank Dr. Sheila Collins for advice and critical reading of the manuscript. We also thank Drs. Michael S. Brown and Joseph L. Goldstein for kindly providing us *SREBP-1c* promoter constructs and Dr. Jiahua Han for p38 α and MKK6E constructs.

REFERENCES

- Cooper, A. D., and Ellsworth, J. L. (1996) *Hepatology* **1**, 92–130
- Accili, D. (2004) *Diabetes* **53**, 1633–1642
- Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) *J. Clin. Investig.* **109**, 1125–1131
- Eberle, D., Hegarty, B., Bossard, P., Ferre, P., and Foulle, F. (2004) *Biochimie (Paris)* **86**, 839–848
- Hua, X., Sakai, J., Ho, Y. K., Goldstein, J. L., and Brown, M. S. (1995) *J. Biol. Chem.* **270**, 29422–29427
- Hua, X., Wu, J., Goldstein, J. L., Brown, M. S., and Hobbs, H. H. (1995) *Genomics* **25**, 667–673
- Miserez, A. R., Cao, G., Probst, L. C., and Hobbs, H. H. (1997) *Genomics* **40**, 31–40
- Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L. (1997) *J. Clin. Investig.* **99**, 846–854
- Horton, J. D., Shah, N. A., Warrington, J. A., Anderson, N. N., Park, S. W., Brown, M. S., and Goldstein, J. L. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 12027–12032
- Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L., and Shimano, H. (1998) *J. Clin. Investig.* **101**, 2331–2339
- Shimano, H., Horton, J. D., Hammer, R. E., Shimomura, I., Brown, M. S., and Goldstein, J. L. (1996) *J. Clin. Investig.* **98**, 1575–1584
- Horton, J. D., Shimomura, I., Ikemoto, S., Bashmakov, Y., and Hammer, R. E. (2003) *J. Biol. Chem.* **278**, 36652–36660
- Shimomura, I., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) *J. Clin. Investig.* **99**, 838–845
- Lee, S. J., Sekimoto, T., Yamashita, E., Nagoshi, E., Nakagawa, A., Imamoto, N., Yoshimura, M., Sakai, H., Chong, K. T., Tsukihara, T., and Yoneda, Y. (2003) *Science* **302**, 1571–1575
- Nagoshi, E., and Yoneda, Y. (2001) *Mol. Cell. Biol.* **21**, 2779–2789
- Horton, J. D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 5987–5992
- Foretz, M., Pacot, C., Dugail, I., Lemarchand, P., Guichard, C., Le Liepvre, X., Berthelie-Lubrano, C., Spiegelman, B., Kim, J. B., Ferre, P., and Foulle, F. (1999) *Mol. Cell. Biol.* **19**, 3760–3768
- Azzout-Marniche, D., Becard, D., Guichard, C., Foretz, M., Ferre, P., and Foulle, F. (2000) *Biochem. J.* **350**, 389–393
- Lin, J., Yang, R., Tarr, P. T., Wu, P. H., Handschin, C., Li, S., Yang, W., Pei, L., Uldry, M., Tontonoz, P., Newgard, C. B., and Spiegelman, B. M. (2005) *Cell* **120**, 261–273
- Rincon, M., Conze, D., Weiss, L., Diehl, N. L., Fortner, K. A., Yang, D., Flavell, R. A., Enslen, H., Whitmarsh, A., and Davis, R. J. (2000) *Immunol. Cell Biol.* **78**, 166–175
- Dong, C., Davis, R. J., and Flavell, R. A. (2002) *Annu. Rev. Immunol.* **20**, 55–72
- Zarubin, T., and Han, J. (2005) *Cell Res.* **15**, 11–18
- Cao, W., Medvedev, A. V., Daniel, K. W., and Collins, S. (2001) *J. Biol. Chem.* **276**, 27077–27082
- Cao, W., Daniel, K. W., Robidoux, J., Puigserver, P., Medvedev, A. V., Bai, X., Floering, L. M., Spiegelman, B. M., and Collins, S. (2004) *Mol. Cell. Biol.* **24**, 3057–3067
- Fan, M., Rhee, J., St-Pierre, J., Handschin, C., Puigserver, P., Lin, J., Jaeger, S., Erdjument-Bromage, H., Tempst, P., and Spiegelman, B. M. (2004) *Genes Dev.* **18**, 278–289
- Akimoto, T., Pohnert, S. C., Li, P., Zhang, M., Gumbs, C., Rosenberg, P. B., Williams, R. S., and Yan, Z. (2005) *J. Biol. Chem.* **280**, 19587–19593
- Cao, W. H., Collins, Q. F., Becker, T. C., Robidoux, J., Lupo, E. G., Jr., Xiong, Y., Daniel, K., Floering, L., and Collins, S. (2005) *J. Biol. Chem.* **280**, 42731–42737
- Collins, Q. F., Xiong, Y., Lupo, E. G., Jr., Liu, H. Y., and Cao, W. (2006) *J. Biol. Chem.* **281**, 24336–24344
- Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. (2000) *Genes Dev.* **14**, 2819–2830
- Seglen, P. O. (1979) *J. Toxicol. Environ. Health* **5**, 551–560
- Kedderis, G. L., Argenbright, L. S., and Miwa, G. T. (1988) *Toxicol. Appl. Pharmacol.* **93**, 403–412
- Lerin, C., Montell, E., Nolasco, T., Clark, C., Brady, M. J., Newgard, C. B., and Gomez-Foix, A. M. (2003) *Diabetes* **52**, 2221–2226
- Xu, J., Teran-Garcia, M., Park, J. H., Nakamura, M. T., and Clarke, S. D. (2001) *J. Biol. Chem.* **276**, 9800–9807
- Shimabukuro, M., Koyama, K., Chen, G., Wang, M.-Y., Trieu, F., Lee, Y., Newgard, C. B., and Unger, R. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 4637–4641
- Ruderman, N., Aoki, T., and Cahill, G. (1976) *Gluconeogenesis: Its Regulation in Mammalian Species*, pp. 515–532, John Wiley & Sons, Inc., New York
- Spector, M. S., Auer, K. L., Jarvis, W. D., Ishac, E. J., Gao, B., Kunos, G., and Dent, P. (1997) *Mol. Cell. Biol.* **17**, 3556–3565
- Amemiya-Kudo, M., Shimano, H., Yoshikawa, T., Yahagi, N., Hasty, A. H., Okazaki, H., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Sato, R., Kimura, S., Ishibashi, S., and Yamada, N. (2000) *J. Biol. Chem.* **275**, 31078–31085
- Chen, G., Liang, G., Ou, J., Goldstein, J. L., and Brown, M. S. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101**, 11245–11250
- Cagen, L. M., Deng, X., Wilcox, H. G., Park, E. A., Raghoebar, R., and Elam, M. B. (2005) *Biochem. J.* **385**, 207–216
- Harris, R. A., Mapes, J. P., Ochs, R. S., Crabb, D. W., and Stropes, L. (1979) *Adv. Exp. Med. Biol.* **111**, 17–42

41. Sweeney, G., Somwar, R., Ramlal, T., Volchuk, A., Ueyama, A., and Klip, A. (1999) *J. Biol. Chem.* **274**, 10071–10078
42. Suzuki, T., Hiroki, A., Watanabe, T., Yamashita, T., Takei, I., and Umezawa, K. (2001) *J. Biol. Chem.* **276**, 27511–27518
43. Konrad, D., Bilan, P. J., Nawaz, Z., Sweeney, G., Niu, W., Liu, Z., Antonescu, C. N., Rudich, A., and Klip, A. (2002) *Diabetes* **51**, 2719–2726
44. Somwar, R., Koterski, S., Sweeney, G., Sciotti, R., Djuric, S., Berg, C., Trevillyan, J., Scherer, P. E., Rondinone, C. M., and Klip, A. (2002) *J. Biol. Chem.* **277**, 50386–50395
45. Bazuine, M., Ouwers, D. M., Gomes de Mesquita, D. S., and Maassen, J. A. (2003) *Eur. J. Biochem.* **270**, 3891–3903
46. Kramer, D. K., Al-Khalili, L., Perrini, S., Skogsberg, J., Wretenberg, P., Kannisto, K., Wallberg-Henriksson, H., Ehrenborg, E., Zierath, J. R., and Krook, A. (2005) *Diabetes* **54**, 1157–1163
47. Richter, E. A., Nielsen, J. N., Jorgensen, S. B., Frosig, C., Birk, J. B., and Wojtaszewski, J. F. (2004) *Proc. Nutr. Soc.* **63**, 211–216
48. McGee, S. L., and Hargreaves, M. (2004) *Diabetes* **53**, 1208–1214
49. McGee, S. L., and Hargreaves, M. (2006) *Clin. Exp. Pharmacol. Physiol.* **33**, 395–399
50. Puigserver, P., Rhee, J., Lin, J., Wu, Z., Yoon, J., Zhang, C., Krauss, S., Mootha, V., Lowell, B., and Spiegelman, B. (2001) *Mol. Cell* **8**, 971
51. Leone, T. C., Lehman, J. J., Finck, B. N., Schaeffer, P. J., Wende, A. R., Boudina, S., Courtois, M., Wozniak, D. F., Sambandam, N., Bernal-Mizrachi, C., Chen, Z., Holloszy, J. O., Medeiros, D. M., Schmidt, R. E., Saffitz, J. E., Abel, E. D., Semenkovich, C. F., and Kelly, D. P. (2005) *PLoS Biol.* **3**, e101
52. Herzig, S., Hedrick, S., Morantte, I., Koo, S. H., Galimi, F., and Montminy, M. (2003) *Nature* **426**, 190–193
53. Xue, B., Coulter, A., Rim, J. S., Koza, R. A., and Kozak, L. P. (2005) *Mol. Cell. Biol.* **25**, 8311–8322
54. Shimano, H. (2001) *Prog. Lipid Res.* **40**, 439–452
55. Kotzka, J., Muller-Wieland, D., Koponen, A., Njamen, D., Kremer, L., Roth, G., Munck, M., Knebel, B., and Krone, W. (1998) *Biochem. Biophys. Res. Commun.* **249**, 375–379
56. Kotzka, J., Muller-Wieland, D., Roth, G., Kremer, L., Munck, M., Schurmann, S., Knebel, B., and Krone, W. (2000) *J. Lipid Res.* **41**, 99–108
57. Kotzka, J., Lehr, S., Roth, G., Avci, H., Knebel, B., and Muller-Wieland, D. (2004) *J. Biol. Chem.* **279**, 22404–22411
58. Lin, J., Tarr, P. T., Yang, R., Rhee, J., Puigserver, P., Newgard, C. B., and Spiegelman, B. M. (2003) *J. Biol. Chem.* **278**, 30843–30848
59. Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelman, G., Stafford, J., Kahn, C. R., Granner, D. K., Newgard, C. B., and Spiegelman, B. M. (2001) *Nature* **413**, 131–138
60. Herzig, S., Long, F., Jhala, U. S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., Spiegelman, B., and Montminy, M. (2001) *Nature* **413**, 179–183

p38 Mitogen-activated Protein Kinase Plays an Inhibitory Role in Hepatic Lipogenesis

Yan Xiong, Qu Fan Collins, Jie An, Edgar Lupo, Jr., Hui-Yu Liu, Delong Liu, Jacques Robidoux, Zhenqi Liu and Wenhong Cao

J. Biol. Chem. 2007, 282:4975-4982.

doi: 10.1074/jbc.M606742200 originally published online December 17, 2006

Access the most updated version of this article at doi: [10.1074/jbc.M606742200](https://doi.org/10.1074/jbc.M606742200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 57 references, 31 of which can be accessed free at <http://www.jbc.org/content/282/7/4975.full.html#ref-list-1>