Free fatty acid (FFA) is believed to be a major environmental factor linking obesity to Type II diabetes. We have recently reported that FFA can induce gluconeogenesis in hepatocytes through p38 mitogen-activated protein kinase (p38). In this study, we have investigated the role of p38 in oleate-induced hepatic insulin resistance. Our results show that a prolonged treatment of primary hepatocytes with oleate blunted insulin suppression of hepatic gluconeogenesis, and decreased insulin-induced phosphorylation of Akt in a p38-dependent manner. Reduction of the insulin-induced Akt phosphorylation by oleate correlated with activation of p38. In the presence of p38 inhibition, prolonged exposure of hepatocytes to oleate failed to reduce insulin-stimulated phosphorylation of Akt. An siRNA against p38α prevented oleate suppression of the insulin-induced phosphorylation of Akt. Furthermore, a prolonged exposure of hepatocytes to oleate decreased insulin-induced tyrosine phosphorylation of IRS1/2, while slightly increasing serine phosphorylation of IRS. The decrease of insulin-stimulated tyrosine phosphorylation of IRS1/2 in hepatocytes by oleate was reversed by the inhibition of p38. We further show that a prolonged exposure of primary hepatocytes to oleate elevated the protein level of the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) gene in a p38-dependent manner, but had no effect on the mRNA level of PTEN. Knocking down the PTEN gene prevented oleate to inhibit insulin activation of Akt and insulin suppression of gluconeogenesis. Together, results from this study demonstrate a critical role for p38 in oleate-induced hepatic insulin resistance.

Plasma levels of free fatty acids (FFAs) are usually increased in obese subjects, and FFAs are considered as a causative link between obesity and Type II diabetes (1–5). A consequence of increased levels of FFA is insulin resistance. Insulin resistance is not only a strong risk factor for cardiovascular diseases (4, 6), but may also eventually lead to Type II diabetes (7), which is currently a major health problem of industrialized countries and tends to increase in its incidence and economic cost because of the increasing trend of obesity. Insulin resistance in liver, adipose tissue, and skeletal muscles plays a central role in the development of Type II diabetes. Specifically, insulin resistance in liver leads to an increased glucose production from hepatic gluconeogenesis, while insulin resistance in adipose tissue and skeletal muscles decreases uptake, utilization, and storage of glucose in these tissues (8, 9). When insulin production from pancreatic islets fails to overcome the insulin resistance, hyperglycemia ensues to announce the presence of frank Type II diabetes.

Hepatic insulin resistance is a powerful promoter of glucose production from liver through gluconeogenesis. The unregulated hepatic gluconeogenesis is a major contributor to hyperglycemia in both Types I and II diabetes (10). Hyperglycemia can damage every organ system by inflaming and clogging blood vessels, which consequently results in heart attacks, strokes, renal failure, blindness, and amputations (11, 12), etc. Equally important, hepatic insulin resistance causes hyperlipidemia, which can further lead to or aggravate global insulin resistance and directly contributes to the development of cardiovascular disorders and other complications related to diabetes (8, 10). Hepatic insulin resistance is tightly correlated with plasma levels of FFA. For example, chronic elevation of plasma FFA in obesity is almost always accompanied by hepatic insulin resistance (7, 8). Acute increase of plasma levels of FFA via lipid infusion can also lead to hepatic insulin resistance (8, 13). The exact mechanism by which FFA induces hepatic insulin resistance has been intensively studied, but much remains undefined.

Elevation of plasma FFA levels is known to activate a series of stress- and inflammation-related kinases, including at least

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[1] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2.

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**Prolonged Treatment of Primary Hepatocytes with Oleate Induces Insulin Resistance through p38 Mitogen-activated Protein Kinase**

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JNK, IKKβ/NF-κB, PKC, and p38 (14). All these kinase systems have been shown to be able to mediate fat-induced insulin resistance in various cellular and tissue types (14, 15). For example, deletion of the JNK1 gene in mouse models (JNK1−/−) can slow down high fat diet-induced global insulin resistance (16). The predominant alteration in JNK1−/− mice is the reduced adipose depot, indicating that the primary tissue affected by JNK is adipose tissue, but not liver (16). Deletion of the IKKβ gene from liver can also prevent high fat diet-induced global and hepatic insulin resistance, while liver-specific expression of the IKKβ gene can promote high fat diet-induced hepatic and global insulin resistance (17, 18). Therefore, it is clear that the IKKβ/NF-κB signaling pathway can play a role in fat-induced hepatic insulin resistance. Some PKC isoforms such as PKCe has been shown to be able to mediate FFA-induced insulin resistance in adipocytes (19). FFA can activate PKCβ in isolated hepatocytes and liver (13, 20). Furthermore, PKCβ is probably involved in FFA-induced insulin resistance (13) and FFA-induced decrease of insulin binding in hepatocytes (21). Finally, p38 has been shown to mediate FFA-induced insulin resistance in various cell types (22–25), but its role in hepatic insulin resistance is unclear.

Because desaturation of saturated FFAs such as stearate into monounsaturated oleate by stearoyl-CoA desaturase-1 (SCD1) is necessary for the onset of high fat diet-induced hepatic insulin resistance (26), and oleate is the most abundant FFA in the plasma (27, 28), we have investigated the role of p38 in oleate-induced insulin resistance in isolated hepatocytes in this study. Our results show that a prolonged exposure of primary hepatocytes to oleate can blunt insulin suppression of hepatic glucose production via gluconeogenesis through p38. We further show that oleate can activate p38 in isolated hepatocytes, and activation of p38 is required for oleate to reduce insulin-stimulated phosphorylation of Akt and tyrosine phosphorylation of IRS1/2. Finally, we have observed that PTEN plays a critical role in oleate-induced hepatic insulin resistance.

MATERIALS AND METHODS

Reagents—Olate and palmitate, fatty acid-free albumin from bovine serum, antibody against phospho-IRS-1(Tyr612), and the ascorbic acid containing the anti-β-actin monoclonal antibody were from Sigma-Aldrich. Antibodies against total/phospho-p38, total/phospho-Akt, total/phospho-PTEN, and phosphotyrosine (p-Tyr-100) were from Cell Signaling Technology (Danvers, MA). Antibodies against insulin receptor substrate-1 (IRS-1) (C-20) and IRS-2 (H-205) and the siRNA against p38α and the scrambled siRNA were from Santa Cruz Biotechnology. The antibody against phosphoserine/threonine (clone 22a) was from BD Biosciences (San Jose, CA). SB202190 and SB203580 were purchased from Calbiochem. The siRNA against PTEN (cat. no. 15034) and related scrambled siRNA (cat. no. 4635) were from Ambion (Austin, TX). Lipofectamine TM 2000 Transfection Reagents were from Invitrogen. Both lactate dehydrogenase (LDH) assay kit and Cell Death Detection ELISA plus kit were from Roche Applied Science (Indianapolis, IN). Other materials were all obtained commercially and are of analytical quality.

Isolation of Hepatocytes—Primary hepatocytes were isolated from C57BL/6 mice as previously described (20, 29, 30). All mice used in the present study for isolation of hepatocytes were fed normal chow diet under a regular schedule unless otherwise noted. Briefly, under anesthesia with pentobarbital (IP, 50 mg/kg body weight), livers were perfused with Ca2+−/−-free Hanks’ Balanced Solution (Invitrogen) at 5 ml/min for 8 min, followed by continuous perfusion with serum-free Williams’ Medium E containing collagenase (Warthington, Type II, 50 units/ml) (Invitrogen) supplemented with 10 mM HEPES for 12 min. Hepatocytes were harvested and purified with Percoll. The viability of hepatocytes was examined with trypan blue exclusion. Only cell isolates with viability over 95% were used. Hepatocytes were inoculated into collagen-coated plates (5 × 105 cells/well in 6-well plates and 1.25 × 106 cell per well in 24-well plates) in Williams’ Medium E with 10% FBS, and were incubated for 24 h before experimentation. All studies were approved by The Hamner Institutes for Health Sciences Animal Care and Use Committee and complied with guidelines from the United States National Institutes of Health.

Olate Preparation and Treatment—Olate was freshly prepared from a 50 mM stock solution in methanol. Specifically, 0.5 mM oleate and 0.1 mM BSA were added to the pre-warmed culture media (37 °C), and mixed by vortexing for 1 min. The media with both oleate and BSA was then used to replace the media in the cell culture immediately. Lower concentrations of oleate/BSA were prepared by adding an equal volume of pre-warmed media. BSA was prepared from a 10× stock solution. Equal amounts of methanol were added into the control cells in each experiment.

Measurement of Glucose Production in Primary Hepatocytes—Primary hepatocytes were isolated from mice, which had been fasted for 24 h to deplete glycogen in liver. Glucose production from primary hepatocytes were measured as previously described (20, 31). Briefly, cells were incubated in Williams’ Medium E supplemented with 10% fetal bovine serum and 0.5 mM oleate for 16 h, and then were washed three times with warm PBS to remove glucose. Subsequently, cells were pre-treated with insulin, followed by stimulation with cAMP/dexamethasone in glucose-free Dulbecco’s modified Eagle’s medium containing gluconeogenic substrates (2 mM sodium pyruvate). Glucose concentrations were determined with a glucose assay kit from Roche Applied Science (cat. no. 0716251) and normalized to protein concentrations. The total glucose production was derived from both glycolysis and gluconeogenesis. Glucose production from gluconeogenesis was measured in the absence of a gluconeogenic substrate. The amount of glucose production by gluconeogenesis is defined as the difference between total glucose production and glycolysis.

Apoptosis Assay—Using a Cell Death Detection ELISA plus kit, levels of cellular apoptosis were quantified by measuring cytoplasmic DNA-histone nucleosome complexes generated during apoptotic DNA fragmentation. In brief, hepatocytes were incubated with oleate or palmitate for 16 h as noted, and then were lysed with the lysis buffer provided by the assay kit. The supernatants of the lysisates were incubated with biotinylated antibodies against histone and peroxidase (POD)-labeled antibodies against DNA in streptavidin-coated microplates for 2 h. Plates were washed to remove
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Because previous studies have suggested a role for p38 in fat-induced insulin resistance in vivo (41–43), and we have previously described a role for p38 in FFA-induced hepatic gluconeogenesis into primary mouse hepatocytes by reverse transfection with Lipo-ffectamine™ 2000 Transfection Reagents. Briefly, the siRNA transfection mixture was applied to collagen-coated 6-well plates right before the plating of primary hepatocytes in culture media without antibiotics. After 24–48 h, cells were treated with oleate together with BSA for 16 h prior to the treatment with insulin as noted.

Real-time PCR—Total RNA was extracted from hepatocytes with an RNase Mini Kit (Qiagen), and reverse-transcribed into cDNA. The cDNA was quantified by TaqMan® Real-time PCR with specific probes and primers from Applied Biosciences, and normalized to levels of GAPDH.

Statistical Analysis—Data are presented as mean ± S.E. of at least three independent experiments. Data were compared by Student’s t test or one-way ANOVA analysis using GraphPad Prism version 4.0 for Windows (San Diego, CA). Differences at values of p < 0.05 were considered significant.

RESULTS

Prolonged Exposure of Hepatocytes to Oleate Blunts Insulin Suppression of Gluconeogenesis in a p38-dependent Manner—Insulin is the predominant suppressor of hepatic gluconeogenesis, and this suppression is blunted in type II diabetes (reviewed in Refs. 10 and 34). It is known that plasma levels of FFA in diabetes are almost always increased, and FFA can induce insulin resistance in various cell types (22–25). Lipid infusion in animal models and in humans can also induce hepatic insulin resistance (8, 13, 35–40). However, the direct role of FFA in the development of insulin resistance in isolated hepatocytes has not been defined. Oleate is the most abundant FFA in the plasma (27, 28), and in humans can also induce hepatic insulin resistance (8, 13, 35–40). Because previous studies have suggested a role for p38 in fat-induced insulin resistance in vivo (41–43), and we have previously described a role for p38 in FFA-induced hepatic gluconeogenesis
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(20), we examined the role of p38 in oleate-induced hepatic insulin resistance. As shown in Fig. 1B, a preincubation of hepatocytes with a p38 inhibitor, SB203580, significantly reduced the capability of oleate to blunt the insulin suppression of hepatic gluconeogenesis. These results indicate that p38 plays a critical role in oleate induction of hepatic insulin resistance.

To examine whether the exposure to oleate was toxic to hepatocytes, levels of LDH release and apoptosis in hepatocytes were measured. As shown in Fig. 1, C and D, oleate did not cause significant cytotoxicity and apoptosis at concentrations lower than 1 mM while palmitate caused obvious toxicity and apoptosis at concentrations higher than 0.2 mM.

Prolonged Exposure of Primary Hepatocytes to Oleate Decreases Insulin-induced Activation of Akt—Insulin suppresses hepatic gluconeogenesis through activation of Akt (reviewed in Refs. 10 and 44). To determine the mechanism by which oleate blunted insulin suppression of hepatic gluconeogenesis, isolated primary hepatocytes were incubated with increasing amount of oleate for 16 h, followed by treatment with insulin for 15 min. Levels of Akt phosphorylation were then measured. As shown in Fig. 2, insulin stimulated phosphorylation of Akt as expected, and this stimulation was decreased by the prolonged incubation with oleate in a dose-dependent manner. Together, these results indicate that a prolonged exposure to oleate can decrease insulin-induced activation of Akt in hepatocytes.

Oleate Stimulates p38 Phosphorylation in Primary Hepatocytes—Previous studies have shown that p38 mediates FFA-induced insulin resistance in several non-hepatocyte cell types (22–25), and we have recently shown that FFA can activate p38 in primary hepatocytes (20). Therefore, we postulated that p38 might play a role in oleate-induced hepatic insulin resistance. To test this hypothesis, primary hepatocytes were treated with increasing amount of oleate in the presence or absence of insulin, followed by measurements of p38 phosphorylation. As shown in Fig. 3, p38 phosphorylation was increased by oleate, and the effect was not influenced by insulin. Insulin itself did not initiate detectable phosphorylation of p38.

p38 Mediates Oleate Suppression of Insulin-induced Activation of Akt—To determine whether the function of oleate in blunting insulin-induced activation of Akt is linked to oleate activation of p38, we took the advantage of chemical inhibitors of p38. Both SB203580 and SB202190 are potent and cell-permeable inhibitors of p38, and they do not suppress other MAP kinases including ERK1/2 and JNK even at 100 μM (45, 46). Primary mouse hepatocytes were pretreated with either SB203580 or SB202190 for 30 min at indicated concentrations prior to the treatment with the SB compound and oleate for 16 h. Cells were subsequently treated with insulin, followed by measurements of Akt phosphorylation. As shown in Fig. 4, insulin stimulation of Akt phosphorylation was blunted by prolonged incubation with oleate. However, the reduction of Akt phosphorylation by oleate were reversed by the inhibition of p38 with either SB203580 (Fig. 4, A and B) or SB202190 (Fig. 4, C and D) in a concentration-dependent manner. Together, these results indicate that activation of p38 is necessary for the development of oleate-induced insulin resistance in hepatocytes.

p38α Is a Critical Mediator for Oleate to Block Insulin-induced Activation of Akt—Four p38 isoforms (α, β, γ, and δ) have been described (47). The activity of p38α and -β can be blocked by SB203580 but not SB202190 (48). Thus, we examined the role of p38α in oleate-induced insulin resistance in hepatocytes. As shown in Fig. 5, SB203580 suppressed the oleate-induced insulin resistance in a concentration-dependent manner. However, SB202190 failed to block oleate-induced insulin resistance. These results indicate that p38α is a critical mediator for oleate-induced insulin resistance in hepatocytes.

Cytokines including TNF-α and IL-1β were used to induce p38 activation in cultured hepatocytes. As shown in Fig. 6, cytokines induced p38 phosphorylation in a concentration-dependent manner. These results suggest that activation of p38 in response to various stimuli is involved in the development of insulin resistance in hepatocytes.

Oleate’s Role in Hepatic Insulin Resistance—Previous studies have shown that oleate blunts insulin suppression of hepatic gluconeogenesis in several non-hepatocyte cell types (22–25). To determine the mechanism by which oleate blunted insulin suppression of hepatic gluconeogenesis, isolated primary hepatocytes were incubated with increasing amount of oleate for 16 h, followed by treatment with insulin for 15 min. Levels of Akt phosphorylation were then measured. As shown in Fig. 2, insulin stimulated phosphorylation of Akt as expected, and this stimulation was decreased by the prolonged incubation with oleate in a dose-dependent manner. Together, these results indicate that a prolonged exposure to oleate can decrease insulin-induced activation of Akt in hepatocytes.

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and SB202190, while p38γ and -δ are insensitive to these compounds. Because the function of p38 in oleate-induced insulin resistance was blocked by either SB203580 or SB202190 as shown in Fig. 4, the role of p38γ and -δ could be excluded. We have recently shown that p38α mediates FFA-induced hepatic gluconeogenesis (20). Therefore, we examined the role of p38α in FFA-induced hepatic insulin resistance using siRNA. The mRNA levels of the p38α gene were reduced by 20–30 and 70% with 5 nM and 50 nM of the siRNA duplexes against p38α, respectively (Fig. 5A). Although silencing of the p38α gene was not 100% in all three experiments, the reduction of Akt phosphorylation by oleate was reversed significantly by the siRNA (Fig. 5, B and C), but was not affected by the scrambled siRNA. Together, these results indicate that p38α is a critical player in the development of oleate-induced insulin resistance in hepatocytes.

Prolonged Exposure of Hepatocytes to Oleate Decreases Insulin-induced Tyrosine Phosphorylation of IRS1/2 through p38—Upon agonist binding, the intracellular signaling pathway of insulin will be activated through sequential phosphorylation of insulin receptors and IRS proteins at tyrosine residues (48, 49). However, insulin signaling is blunted when IRS phosphorylation occurs at serine residues (48, 49). Therefore, levels of IRS phosphorylation at tyrosine and serine residues can be used as markers to evaluate insulin sensitivity. To further examine the effect of prolonged exposure to oleate on hepatic insulin signaling, levels of IRS phosphorylation at tyrosine and serine residues were estimated by immunoprecipitation and immunoblotting. As shown in Fig. 6A, insulin induced tyrosine phosphorylation of IRS1 and IRS2 in primary hepatocytes as expected. The prolonged treatment of hepatocytes with oleate reduced insulin-initiated tyrosine phosphorylation of both IRS1 and IRS2, while slightly elevated serine phosphorylation of IRS1. Meanwhile, the prolonged treatment of hepatocytes with oleate did not significantly influence the protein level of IRS1. Together, these results indicate that a prolonged exposure of hepatocytes to oleate decreases insulin-induced tyrosine phosphorylation of IRS1/2.

To determine the role of p38 in oleate-mediated reduction in tyrosine phosphorylation of IRSs, p38 activity was blocked prior to the prolonged exposure of primary hepatocytes to oleate. As shown in Fig. 6, B and C, inhibition of p38 was able to prevent oleate to inhibit insulin-induced tyrosine phosphorylation of IRS1. These results further indicate that p38 plays a critical role in oleate-induced hepatic insulin resistance.

Oleate Elevates PTEN Protein Level through p38 in Hepatocytes—A couple of recent studies in vascular endothelial cells have shown that p38 participates in FFA-induced insulin resistance.

![Figure 4](image-url) **FIGURE 4.** p38 activation is required for oleate suppression of insulin-induced phosphorylation of Akt. Mouse primary hepatocytes were isolated and cultured as detailed under “Materials and Methods.” Hepatocytes were pretreated with 1 or 10 μM of p38 inhibitor SB203580 (A and B) or SB 202190 (C and D) as indicated prior to the exposure to oleate for 16 h. Cells were then stimulated with insulin (10 nm) for 15 min. Levels of total and phospho-Akt were measured by immunoblotting with specific antibodies. Results represent mean ± S.E. of three independent experiments. *, p < 0.01 versus all other bars without * (one-way ANOVA).

![Figure 5](image-url) **FIGURE 5.** p38α mediates oleate suppression of insulin-stimulated Akt phosphorylation of Akt. Mouse primary hepatocytes were isolated and cultured as detailed under "Materials and Methods." The siRNAs were introduced into the hepatocytes via transient transfection as noted. After 24 h, cells were exposed to oleate for 16 h, followed by treatment with insulin (10 nm) for 15 min. The expression of p38α was evaluated by immunoblotting with anti-p38 antibodies (A). Total and phospho-Akt were detected by immunoblotting with specific antibodies (B and C). Results represent mean ± S.E. of three independent experiments. *, p < 0.05 versus all other bars without * (one-way ANOVA).
resistance by promoting expression of the PTEN gene (25, 43).
Therefore, we postulated that p38 in hepatocytes might desensitize insulin signaling through PTEN. To test this hypothesis, primary hepatocytes were treated with either SB203580 or an siRNA against the p38 gene, and then exposed to oleate for 16 h, followed by measurement of the PTEN protein. As shown in Fig. 7A, treatment of hepatocytes with oleate indeed increased the protein level of PTEN, and the increase was blocked by the inhibition of p38 with either SB203580 or the siRNA against the p38 gene. These results suggest that oleate can enhance PTEN protein level in a p38-dependent manner.

We next examined the effect of prolonged exposure of hepatocytes to oleate on the mRNA level of PTEN. Surprisingly, oleate had no effect on the mRNA level of PTEN (Fig. 7B). These results indicate that the effect of oleate on PTEN protein shown in Fig. 7A was not at the mRNA level. Since it is established that PTEN protein can be stabilized by phosphorylation at serine 380, threonine 382, and threonine 383 of its C-terminal (reviewed in Ref. 50), phosphorylation at these sites was examined. As shown in Fig. 7C, phosphorylation of PTEN at these sites was indeed enhanced by oleate.

To determine whether PTEN is a mediator of oleate-induced insulin resistance, the PTEN gene in hepatocytes was knocked down by a siRNA against the PTEN gene (Fig. 8A). When the PTEN gene was silenced, oleate failed to attenuate the insulin induction of Akt phosphorylation (Fig. 8B). Further- more, silencing the PTEN gene prevented oleate to reduce the insulin suppression of gluconeogenic gene transcription including G6Pase and PEPCK (Fig. 8C). Together, these results indicate a critical role for PTEN in oleate-induced hepatic insulin resistance.

DISCUSSION

Many stress- and inflammation-related kinases such as JNK, NF-kB, and PKCs have been shown to mediate fat-induced
hepatic insulin resistance. Here we show in cultured primary hepatocytes that p38 plays an important role in the development of hepatic insulin resistance.

p38 can be a mediator of FFA-induced insulin resistance in various cell types. It has recently been shown in endothelial cells that FFA can induce insulin resistance and consequently lead to a decrease in activity of the endothelial nitric-oxide synthase (eNOS) (25). Blockade of p38 can prevent FFA-induced decrease in activities of both Akt and eNOS. It is believed that p38 participates in FFA-induced insulin resistance by promoting expression of the PTEN gene (25). These observations are further supported by some earlier studies, which showed that blockade of p38 could actually prevent both activation of p38 on the lumen surface of arteries and development of high fat diet-induced hypertension in mice (51). It has also been shown that p38 plays a critical role in FFA-induced insulin resistance in adipocytes and consequently reduces glucose uptake by adipocytes. In this case, p38 accomplishes its role by decreasing expression of IRS1/2 genes (22). Studies have also implicated that p38 may play a role in the development of insulin resistance in skeletal muscles in type II diabetes, which is often associated with increased plasma levels of FFA (52). In this study, we show that p38 is a mediator of oleate-induced insulin resistance in isolated hepatocytes through altering levels of IRS phosphorylation and PTEN protein. Together with previous reports from us and others, it appears that p38 cannot only directly stimulate insulin resistance (17). The liver-specific activation of NF-κB through transgenic expression of IKKβ, an activator of NF-κB signaling pathway, promotes hepatic and global insulin resistance, while liver-specific knock-out of the IKKβ gene can protect mice from high fat diet-induced hepatic insulin resistance (17, 18). Similarly, PKCδ has clearly been shown to be involved in FFA-induced hepatic insulin resistance (13). We have also shown that levels of PKCδ phosphorylation is increased both in isolated hepatocytes treated with FFA and in liver of mice with high fat diet (20). Therefore, it is clear that all JNK, NF-κB, certain isoforms of PKC, and p38 are mediators of fat-induced hepatic insulin resistance. In order to determine the relative contribution of these kinases, we tried to block activation these kinases one at a time prior to the prolonged exposure of hepatocytes to oleate, followed by measurements of insulin-induced phosphorylation of Akt. Surprisingly, blockade of any one of these kinases alone prevented oleate-induced hepatic insulin resistance, indicating that these kinases are somehow functionally connected together either in a single linear cascade or as a complex interacted work net. The exact mechanism by which these kinases functionally depend on each other remains unknown. It is also unknown whether the role of each kinase system is compartmentalized. Because our current understand-

3 W. Cao, unpublished data.
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ing of these kinases is mostly based on various random studies, it may be helpful to map out the dynamic signaling net of FFA in hepatocytes by systematically identify all the kinases activated by the treatment of FFA such as oleate at different time points.

In conclusion, this study has identified p38, as a player in oleate-induced hepatic insulin resistance. Our results show that a prolonged exposure of hepatocytes to oleate, which is similar to the situation seen in obesity and diabetes, can induce insulin resistance by activating p38. p38 desensitizes the insulin signaling pathway by altering levels of both IRS phosphorylation and PTK6 protein. Our finding is consistent with the notion that the presence of monounsaturated FFA such as oleate is necessary for fat-induced hepatic insulin resistance (26). However, the signaling pathway of oleate and the relative contribution of p38 versus other known players such as JNK, NF-κB, and PKCs to FFA-induced hepatic insulin resistance remain to be determined in future studies.

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