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Saturated Fatty Acids Inhibit Hepatic Insulin Action by Modulating Insulin Receptor Expression and Post-receptor Signalling

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Free fatty acids (FFAs) are proposed to play a pathogenic role in both peripheral and hepatic insulin resistance. We have examined the effect of saturated FFA on insulin signalling (100 nM) in two hepatocyte cell lines. Fao hepatoma cells were treated with physiological concentrations of sodium palmitate (0.25 mM) (16:0) for 0.25–48 h. Palmitate decreased insulin receptor (IR) protein and mRNA expression in a dose- and time-dependent manner (35% decrease at 12 h). Palmitate also reduced insulin-stimulated IR and IRS-2 tyrosine phosphorylation, IRS-2-associated PI 3-kinase activity, and phosphorylation of Akt, p70 S6 kinase, GSK-3 and FOXO1A. Palmitate also inhibited insulin action in hepatocytes derived from wild-type IR (+/+) mice, but was ineffective in IR-deficient (−/−) cells. The effects of palmitate were reversed by triacsin C, an inhibitor of fatty acyl CoA synthases, indicating that palmitoyl CoA ester formation is critical. Neither the non-metabolized bromopalmitate alone nor the medium chain fatty acid octanoate (8:0) produced similar effects. However, the CPT-1 inhibitor (±)-etomoxir and bromopalmitate (in molar excess) reversed the effects of palmitate. Thus, the inhibition of insulin signalling by palmitate in hepatoma cells is dependent upon oxidation of fatty acyl-CoA species and requires intact insulin receptor expression.

Key words: β-oxidation, insulin signalling, insulin resistance, liver, palmitate.

Abbreviations: C16, palmitate; DAG, diacylglycerol; FFA, free fatty acid; IR, insulin receptor; IRS, insulin receptor substrate; PKC, protein kinase C.

Increased plasma concentrations of free fatty acids (FFAs) are associated with many insulin-resistant states, including obesity and type 2 diabetes (DM), and have been proposed to play a pathogenic role in both peripheral and hepatic insulin resistance (*1*). *In vivo* infusion of FFA into both rodents and humans induces insulin resistance in skeletal muscle and increases hepatic glucose production (*2*, *3*). However, the cellular mechanisms for these effects remain unclear. Randle *et al*. (*4*) originally proposed the glucose–FA cycle, in which increased fat oxidation would increase acetyl CoA levels and NADH/NAD+ ratios, decrease pyruvate dehydrogenase activity, increase citrate, inhibit phosphofructokinase, and ultimately, increase glucose-6-phosphate. Increased G-6-P levels would allosterically inhibit hexokinase II activity and glucose oxidation. More recent studies using NMR spectroscopy demonstrate no increase in skeletal muscle G-6-P levels with FA infusions (*5*) and instead support a direct inhibitory effect of FAs on insulin signalling, glucose transport, glycogen synthesis and gene expression (*6–9*). Potential candidates mediating these effects include increased ceramide production (*10*), accumulation of diacylglycerol leading to activation of novel PKCs (*11*), and lipid-induced activation of the hexosamine biosynthetic pathway (*12*) and NF-κB (*9*), among others.

Increasing evidence supports a particularly important role for the liver in the development of insulin resistance. Deletion of the insulin receptor (IR) in liver causes insulin resistance and diabetes in mice (*13*), while deletion of insulin receptor substrate-2 (IRS-2), the substrate expressed abundantly in liver but minimally in muscle, results in both hepatic and whole-body insulin resistance and overt diabetes (*14*). Decreased expression of both IRS-1 and IRS-2 results in hepatic steatosis and systemic insulin resistance (*15*). In addition, high portal vein concentrations of FFA, derived from visceral adipose stores, may increase hepatic glucose production, decrease insulin clearance, increase production of very low density lipoproteins (VLDL) and induce (secondary) peripheral insulin resistance (*16*).

We now demonstrate that a representative abundant long-chain saturated FA, sodium palmitate (C16:0), inhibits the metabolic actions of insulin and attenuates insulin signal transduction in two well-differentiated hepatoma cell lines. This attenuation of insulin signalling by palmitate requires synthesis of palmitoyl CoA, is inhibited by etomoxir, and requires intact IR expression.

MATERIALS AND METHODS

Antibodies and Chemical Reagents—Anti-IR (JD15), anti-IRS-1 (JD287), anti-IRS-2 (JD250) and anti-pY

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(4G10) were generously provided by Drs C. Ronald Kahn and Morris White, Joslin Diabetes Center. Rabbit anti-p85 (06-185), anti-p110α/β anti-phospho-FOXO1A1 and anti-FOXO1A1 antibodies were from Millipore, Billerica, MA. Rabbit anti-phosphoERK was from Promega, Madison, WI, while phosphospecific rabbit anti-Akt (Ser^{473}), rabbit anti-GSK-3α/β (Ser^{21} and Ser^{9}), rabbit anti-IκB-α (Ser^{32}), anti-S70S6 kinase (Thr^{386}) and anti-p38 were from New England Biolabs, Ipswich, MA. Anti-L-FABP antibody was generously provided by Dr Jeffrey Gordon, Washington University School of Medicine. All chemical reagents were purchased from Sigma, St. Louis, MO, unless otherwise indicated. (±) Etomoxir was obtained from Dr H.P.O. Wolf, Im Tal 17, D-784676 Germany (+49 753 397 164). LY379196 was generously provided by Lilly (Indianapolis, IN, USA).

Cell Culture—Well-differentiated rat hepatoma cells (Fao) were grown to 70–80% confluence in RPMI-1640 with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA). For additional experiments, transformed hepatocytes were isolated from wild-type (+/+) and IR-deficient (−/−) mice between $ED_{18.5}$ and post-natal day 1 were generously supplied by Dr Domenico Accili (*17*). Wild-type and IR (−/−) cells were grown at 33°C in αMEM with 4% FBS, 500 nM dexamethasone and 2 mM L-glutamine and differentiated at 37°C in the absence of dexamethasone.

Insulin Stimulation and Western Blotting—Cells were serum-starved in RPMI-1640 (Fao) or αMEM (transformed hepatocytes) with 25 mM HEPES and 0.5% insulin-free BSA (Arnel), pH 7.4 and incubated ± insulin (0.1–100 nM) for 5–30 min. Cells were lysed in 50 mM HEPES, pH 7.4, 140 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Na pyrophosphate, 100 mM NaF, 2 mM EDTA, 10% glycerol, 1% NP-40, 2 mM Na_3VO_4, 2 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 10 mM benzamidine. Lysates were cleared by centrifugation and incubated with the indicated antibody overnight at 4°C. Immunoprecipitates were collected with protein A-sepharose (Pharmacia, Piscataway, NJ), washed, solubilized in Laemmli sample buffer, separated by SDS–PAGE, transferred to nitrocellulose (Millipore), and immunoblotted with the indicated primary antibody. ^{125}I-protein A (ICN) or ECL reagents (NEN) were used for detection; quantification was performed using Molecular Dynamics PhosphorImager (for ^{125}I) or scanning densitometry with ImageQuant software.

Phosphatidylinositol 3-kinase Activity—PI 3-kinase activity was measured by *in vitro* phosphorylation of phosphatidylinositol (PI) in immune complexes as described (*18*).

Cell Fractionation—Cells were washed with ice-cold PBS, harvested into buffer A (20 mM Tris, 2 mM EDTA, 0.5 mM EGTA, 0.3 M sucrose, 1 mM PMSF, 1 mM DTT, 50 μg/ml leupeptin, pH 7.4) and Dounce homogenized for 60 strokes. Samples were spun at 2,500 r.p.m. for 10 min at 4°C. Supernatant was spun at 55,000 r.p.m. for 30 min, and resulting supernatant identified as cytosol fraction. Pellet was resuspended in buffer B (buffer A without sucrose), homogenized for 40 strokes and solubilized with the addition of Triton X-100 to final concentration of 1%. Sample was spun at 55,000 r.p.m. for 30 min, with supernatant identified as membrane fraction.

Preparation of BSA-conjugated FAs—FAs (100 mM, Alltech, Deerfield, IL, USA) were dissolved in 0.1 N sodium hydroxide and heated to 65°C for 1 h, mixed by inversion, and added to 10% FA-free BSA (Boehringer Mannheim, Germany) at 50°C to yield a final 5 mM stock (stored at −20°C).

FA Uptake—After washing cells twice with PBS, RPMI-1640 with 0.5% FA-free BSA and 25 mM HEPES were added to each dish in the absence or presence of BSA (0.5%) (control) or BSA-palmitate (250 μM). The 100 μl aliquots were removed at $t = 0$, 0.25, 0.5, 0.75, 1, 2, 3, 6 and 24 h and replaced with fresh medium. The FA in the conditioned medium was measured (Wako Chemicals, Waco, TX, USA) and normalized for protein.

Hepatic Glucose Production—Fao cells were grown to 80% confluence in 6-cm cultures dishes and incubated overnight in glucose-free RPMI-1640 with 0.5% insulin-free BSA, 25 mM HEPES, 16 mM lactate, and 4 mM pyruvate (*17*). Palmitate–BSA or BSA alone (control) ± 100 nM insulin were added. Medium was removed at $t = 0$, 6 and 12 h; glucose was measured using a spectrophotometric method (Sigma) and normalized for protein.

Glycogen Content—The 80% confluent cells were washed, and RPMI-1640 with 0.5% FA-free BSA and 25 mM HEPES was added to each dish prior to overnight incubation with either BSA or palmitate (250 μM) ± insulin (100 nM). Glycogen content was assessed by PAS staining (Sigma).

Northern Blot Analysis—Total RNA was isolated using Trizol (Gibco). A total of 40 μg were resolved in a formaldahyde/agarose denaturing gel. After washing gel in 10× SSC, RNA was transferred onto nylon membranes (Schleicher and Schuell, Keene, NH) and cross-linked. An α-subunit IR probe was isolated from the plasmid pSG-HIRC by HindIII, SpcI and NdeI digestion (*19*), purified, and labelled with (α-^{32}P) dCTP (NEN). The membrane was hybridized at 65°C for 1.5 h, washed, dried and visualized by autoradiography.

UDP-GlcNAc Assay—Levels of UDP-N-acetylglucosamine were assayed using an HPLC-based assay (*20*).

Statistical Analysis—The paired Student's *t*-test was used to test for significance in all experiments. The results are presented as mean ± SEM, and represent at least three independent experiments. $P < 0.05$ was considered statistically significant.

RESULTS

FA Clearance—ED_{50} for clearance of palmitate from conditioned medium was 1.55 h ($r = 0.97$). At 6 h, the concentration of palmitate in the medium was reduced by 89%; by 24 h, we could not detect any remaining FA (data not shown).

Palmitate Inhibits the Ability of Insulin to Suppress Hepatic Glucose Production—Previous studies have demonstrated that portal FAs may regulate hepatic glucose production (HPG) (*2*). To evaluate the effects of FAs on glucose production and insulin action in the absence of complicating systemic hormonal and metabolic changes, we incubated Fao hepatoma cells, a well differentiated and

insulin-responsive culture model, with palmitate, a highly abundant saturated FA. To measure glucose production, cells were incubated in glucose-free medium containing pyruvate (4 mM) and lactate (16 mM) as gluconeogenic substrates, in the presence or absence of palmitate for 0–12 h. As expected, inclusion of insulin in the incubation medium reduced glucose release into medium by 65% ($P < 0.001$) in BSA-treated cells. However, palmitate exposure reduced the ability of insulin to suppress glucose production by 16% at 12 h ($P < 0.03$). Similarly, palmitate abolished insulin-stimulated glycogen accumulation (2.1-fold increase in BSA, $P < 0.02$, versus no change in FFA, $P < 0.05$ for insulin-stimulated BSA versus FFA).

Palmitate Reduces IR β-subunit Tyrosine-phosphorylation—To determine if palmitate induced abnormalities in insulin signalling, Fao cells were incubated with palmitate or BSA for 12 h and stimulated with insulin. As expected, insulin increased tyrosine phosphorylation of the β-subunit of the IR by 84%. However, pre-treatment with palmitate (250 μM) decreased insulin-stimulated tyrosine phosphorylation by 31% (Fig. 1A). The ability

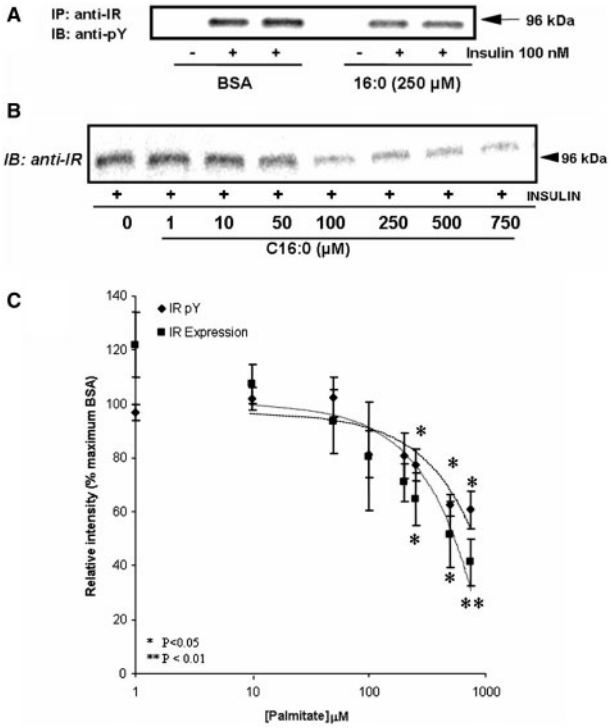

Fig. 1. **Palmitate reduces IR tyrosine phosphorylation and expression.** (A) Fao cells were treated with either BSA or BSA conjugated to palmitate (C16:0, 250 μM) for 12 h, then stimulated with PBS or 100 nM insulin for 30 min. Cell lysates were immunoprecipitated with anti-IR (β-subunit) and immunoblotted with anti-phosphotyrosine (4G10) (pY) antibodies. A representative anti-phosphotyrosine blot is shown. (B) Pre-treatment of cells with palmitate (1–750 μM) (12 h) significantly attenuated the insulin-mediated increase in IR pY. Significant differences in IR pY values were observed at palmitate concentrations ≥250 μM. (C) IR pY and expression, following palmitate (1–1000 μM) treatments (12 h) expressed as percentage of BSA, relative to Fao standard, and represent means ± SEM for three independent experiments. *Significant difference from BSA control ($P < 0.05$).

of palmitate to reduce IR phosphorylation was both time-dependent, reaching significance by 12 h and sustained up to 48 h, and dose-dependent, with significant reductions observed at concentrations ≥ 250 μM. The effect of palmitate (0–750 μM) (12 h) on IR pY is shown in Fig. 1B.

In close parallel with inhibition of tyrosine phosphorylation, expression of the IR β-subunit was also decreased by palmitate in a dose- and time-dependent fashion. Figure 1C describes the effect of palmitate (1–1,000 μM) (12 h) on IR pY and IR expression. Inhibition of IR expression was noted at 6 h following palmitate incubation (40% decrease at 6 h, 60% at 12 h, $P < 0.05$) and was sustained up to 48 h. These effects did not appear to be related to differential membrane solubility of the receptor in FFA-treated cells, as direct SDS lysis of whole cells also yielded a similar decrease in IR expression (data not shown).

Palmitate Reduces Tyrosine Phosphorylation of IRSs IRS-1 and IRS-2—Acute insulin stimulation stimulated tyrosine phosphorylation of the major IRS proteins in the liver, IRS-1 and IRS-2 (data not shown). However, exposure of cells to palmitate decreased insulin-stimulated IRS-2 tyrosine phosphorylation by 22% (250 μM, 12 h, $P = 0.01$) (data not shown). These changes were sustained up to 48 h. Palmitate did not significantly alter IRS-1 tyrosine phosphorylation at 12 h, but decreased IRS-1-dependent signalling by 31% at 48 h ($P < 0.01$).

Palmitate Reduces Association of IRS-1 and IRS-2 with the p85 Regulatory Subunit of PI 3-kinase and Activation of PI 3-kinase—Insulin-mediated tyrosine phosphorylation of IRS-1 and IRS-2 increases their association with the p85 regulatory subunit of PI 3-kinase, activating this essential step for many of insulin's biological activities (*18*). Palmitate (250 μM) significantly reduced association of both IRS-1 (34% reduction, $P < 0.009$) and IRS-2 (29% reduction, $P < 0.02$) with p85 at 12 h (data not shown). Palmitate also inhibited insulin-stimulated IRS-2-associated PI 3-kinase activity, by 45% at 12 h ($P < 0.001$), 35% at 24 h ($P < 0.003$) and 28% at 48 h ($P < 0.001$) (data not shown). Palmitate effects to reduce IRS-1 associated PI 3-kinase activity were delayed, reaching statistical significance at 48 h (31% reduction, $P < 0.003$)—in accord with the delayed effects on tyrosine phosphorylation of IRS-1.

To determine if palmitate affected the association between p85 and the p110 catalytic subunit of PI 3-kinase, we immunoprecipitated lysates with anti-p110α/β antibodies and immunoblotted with anti-p85. Palmitate (250 μM) reduced p85 association with p110 by 56%. This appeared to be accounted for in part by a dose-dependent decrease in p85α protein expression (36% reduction, 250 μM palmitate, data not shown).

Palmitate Inhibits the Ability of Insulin to Stimulate Akt phosphorylation, glycogen synthase kinase 3 *Inactivation, and FOXO Phosphorylation*—Given the importance of the serine/threonine kinase Akt in mediating insulin action on metabolism and transcriptional regulation (*21*), we assessed effects of palmitate on insulin-stimulated phosphorylation of Akt1/2 using phosphospecific (Ser^{473}) antibodies. Palmitate inhibited insulin-stimulated Akt phosphorylation by 61% at 12 h ($P < 0.01$) in a dose-dependent manner (Fig. 2A), decreasing both the

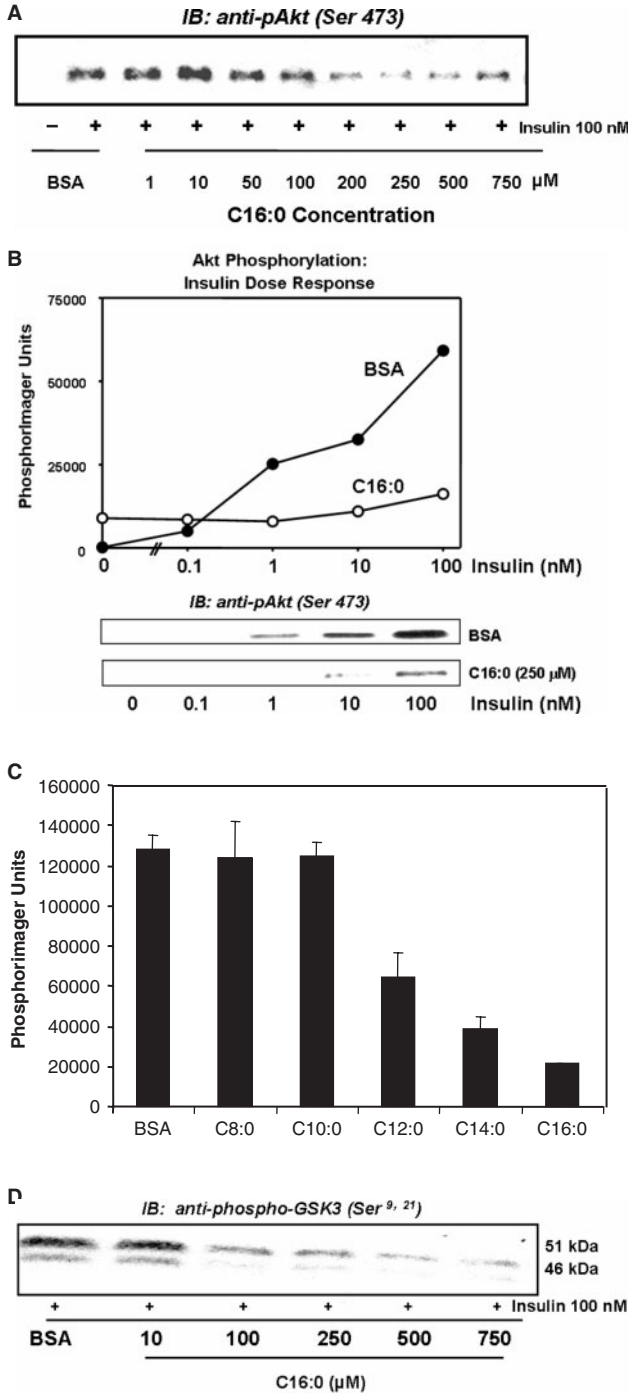

Fig. 2. **Palmitate inhibits insulin-stimulated Akt and GSK-3 phosphorylation.** (A) Fao cells were incubated with either BSA or palmitate (1–750 μM) for 12 h and treated with insulin (0–100 nM) for 30 min. Lysates were immunoblotted with phosphospecific anti-Akt (Ser^{473}). (B) Quantitation of dose-dependent Akt phosphorylation for BSA or palmitate. Representative phospho-Akt blots are provided below the graph. (C) Cells were incubated for 12 h with their respective fatty acid [Caprylic (C8:0); Capric (C10:0); Lauric (C12:0); Myristic (C14:0), Palmitic (16:0)] or BSA and then stimulated with insulin (100 nM) (30 min). Cells were harvested and then lysed. Lysates were immunoblotted with anti-phosphoAkt. Quantification data is presented (D) Representative phospho-specific anti-GSK-3 ($Ser^{9, 21}$) immunoblot.

maximal insulin effect and shifting the insulin dose–response curve to the right (Fig. 2B). We observed no change in Akt expression (not shown). A further series of experiments were conducted to evaluate the potential effect of the FA chain length on Akt phosphorylation. The following FAs were investigated: (i) Caprylic (C8:0), (ii) Capric (C10:0), (iii) Lauric (C12:0), (iv) Myristic (C14:0) and (v) Palmitic (C16:0). In addition, each of the FAs was used at equienergenic concentrations. As expected, the higher the saturation of the FA, the greater the decrease in Akt phosphorylation. (C16 > C14 > C12 > C10 > C8) (Fig. 2C). Key downstream targets of Akt phosphorylated and inactivated in response to insulin include glycogen synthase kinase 3 (GSK-3) and the forkhead family of transcription factors (*22*, *23*). Palmitate significantly reduced the insulin-stimulated phosphorylation of GSK-3 in a dose-dependent manner (65% for 250 μM, $P < 0.05$, Fig. 2D) and FOXO1 by 27% ($P < 0.01$), with no reduction in protein expression (not shown). Similarly, palmitate reduced phosphorylation of p70 S6 kinase, a target of both insulin action and Tor-dependent signalling (data not shown).

Inhibitory Effects of Palmitate do not Extend to the ERK or p38 Pathways—Since insulin resistance in both humans and in Zucker fatty rats is characterized by selective impairment in PI 3-kinase-dependent pathways, with a sparing of ERK pathways (*24*, *25*), we evaluated palmitate effects on insulin stimulation of ERK and p38 kinases. Palmitate augmented basal and insulin-stimulated ERK phosphorylation by 27% at 12 h ($P < 0.01$) (Fig. 3A) and increased p38 phosphorylation by 65% ($P < 0.05$) (Fig. 3B). Pre-incubation with either the ERK inhibitor PD98059 (10 and 50 μM) or the selective p38 inhibitor SB203580 (10 μM), while effective, did not reverse palmitate inhibition of insulin receptor (Fig. 3C) or Akt phosphorylation (data not shown).

Cell Viability and Apoptosis are not Altered by Palmitate—To exclude a toxic effect of palmitate due to decreased membrane integrity or induction of apoptosis, we analysed trypan blue exclusion and activity of cleaved caspase-3 by western blot. There was no effect of palmitate–BSA (250 μM, 12 h) on trypan blue exclusion, caspase-3 activity or cell morphology (data not shown). In addition, adaptive transcription and translation responses were maintained, as indicated by the ability of palmitate (250 μM) to increase the expression of FA-binding protein by 7-fold ($P = 0.02$, not shown), as previously reported (*26*).

Role of Candidate Signalling Cascades in Mediating Effects of Palmitate—In skeletal muscle, FAs increase formation of diacylglycerol, leading to activation of both conventional and novel isoforms of protein kinase C (PKC), including θ, β and δ (*6*, *27*). To assess PKC activation in hepatoma cells, we immunoblotted cytosolic and membrane fractions. Palmitate increased expression and membrane content of PKCδ and ε and phosphorylation of PKCδ, but had no effect on conventional isoform expression or distribution (data not shown). The PKC inhibitor bisindoylmaleimide (10 μM) was effective in reversing the phosphorylation of PKCδ by PMA (1 μM). However, neither bisindoylmaleimide (in doses up to 10 μM) nor the β1-specific inhibitor LY 379196 were effective in

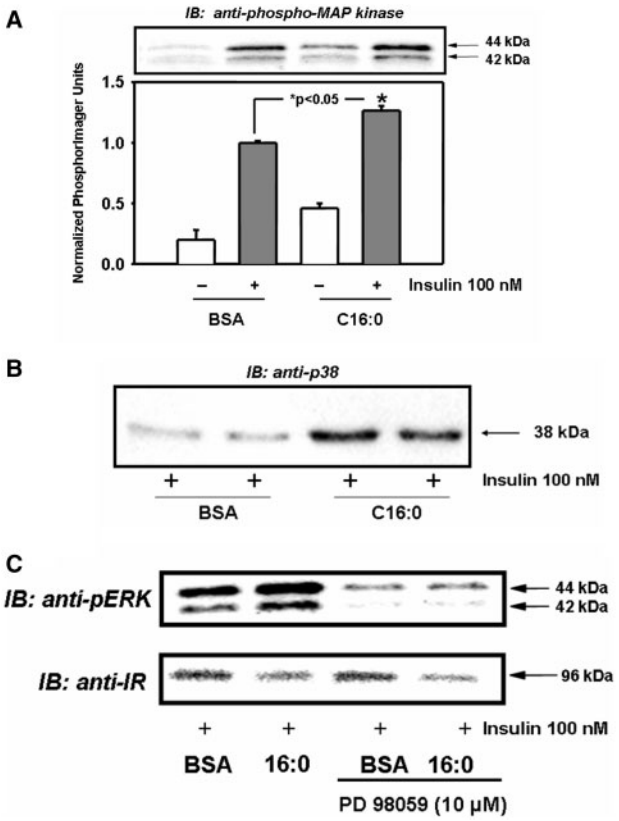

Fig. 3. **Palmitate does not inhibit ERK or p38 phosphorylation.** (A and B) Cell lysates (50 μg) from BSA or palmitate-treated cells stimulated with PBS or insulin were immunoblotted with phosphospecific anti-ERK or anti-p38 antibody (Promega), detected with ECL and quantitated by densitometry. $^{*}P < 0.05$ for BSA versus palmitate. (C) Representative blots are shown for anti-phosphoERK and IR following pre-treatment of cells with MEK inhibitor PD98059 (10 μM) and p38 inhibitor SB203580 (10 μM).

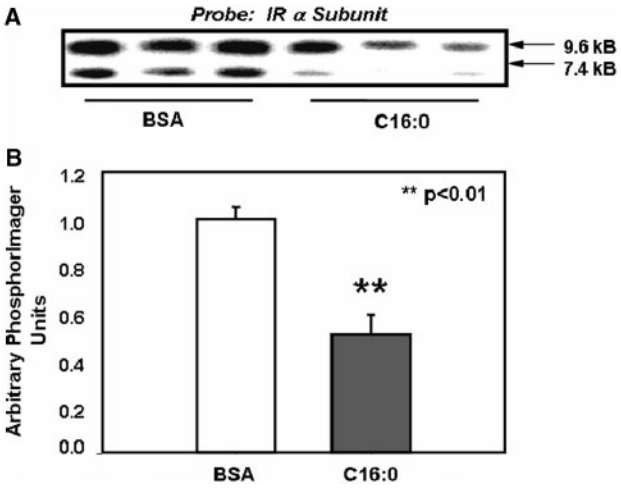

Fig. 4. **Palmitate reduces insulin receptor mRNA expression.** Cells were treated with BSA or palmitate 250 μM for 12 h. (A) A representative northern blot, while (B) quantitative data from three independent experiments. $^{**}P < 0.01$ for C16:0 versus BSA.

blocking the effects of palmitate on insulin action (data not shown).

A serine kinase cascade involving IKK phosphorylation of IκB and subsequent activation of NF-κB has been implicated in lipid-induced insulin resistance in skeletal muscle (*28*). We observed a dose-dependent decrease in phosphorylation of IκB-α (Ser^{32}) in response to palmitate-treated Fao cells stimulated with insulin (100 nM) (data not shown). However, neither 5 mM aspirin, an inhibitor of IKK, nor 18 μM SN50, an inhibitor of NF-κB nuclear translocation and gene expression, were effective in reversing palmitate effects on Akt phosphorylation or IR expression (data not shown).

Both FAs and glucose can mediate nutrient-induced insulin resistance via activation of the hexosamine pathway (*29–31*). To assess activation of this pathway, we measured intracellular levels of UDP-*N*-acetylglucosamine (UDP-GlcNAc) (*20*). Although 2.5 mM glucosamine increased UDP-GlcNAc by 7.5-fold relative to untreated cells (63.4 pmol/μg protein versus 8.5 pmol/μg, $P < 0.001$), as expected, palmitate actually decreased UDP-GlcNAc levels by 63% (palmitate 3.13 pmol/μg, $P = 0.03$). Moreover, pre-treatment of Fao cells with azaserine, an inhibitor of glutamine-fructose amidotransferase 6 phosphate amido transferase (GFAT), the rate-limiting enzyme in the hexosamine pathway, did not reverse palmitate-induced insulin resistance. Similarly, inhibition of the mTor nutrient-sensing pathway by rapamycin was unable to reverse the effects of palmitate (data not shown).

Palmitate Reduces IR Protein and mRNA Expression—To determine whether palmitate effects on downstream insulin signalling were mediated by altered IR expression, we performed northern blot analysis of IR α-subunit expression. As seen in Fig. 4, palmitate treatment for 12 h reduced the expression of IR mRNA by 49% ($P < 0.01$).

Palmitate does not Inhibit Insulin Signalling in IR-deficient Hepatocytes—To determine whether there were additional post-receptor effects of saturated FAs, we evaluated the effect of palmitate in transformed hepatocytes isolated from IR wild-type (+/+) and knockout (–/–) mice (*17*). In wild-type cells, IR protein expression, Akt and GSK-3 phosphorylation were all significantly reduced by palmitate, by 51% ($P < 0.05$), 50% ($P < 0.03$) and 70% ($P < 0.01$), as in Fao. Similarly, IκB-α (Ser^{32}) and p70 S6 kinase phosphorylation were also attenuated by palmitate in (+/+) cells. In $IR^{-/-}$ cells, insulin was able to stimulate IRS-2 phosphorylation, activation of PI 3-kinase and phosphorylation of Akt and GSK-3, via the IGF-1 receptor (*17*, *32*). However, incubation of *IR* (–/–) cells with palmitate did not attenuate insulin action at any level, including Akt (Fig. 5A), p70 S6 kinase (Fig. 5B) or IκB-α phosphorylation (Fig. 5C).

Long-chain acyl CoA Synthesis and β-oxidation are Required for the Inhibitory Effects of Palmitate on Insulin Action—To determine whether palmitate effects were mediated by palmitate itself or palmitoyl CoA, cells were incubated with triacsin C, an inhibitor of acyl CoA synthetase. Triacsin C completely abolished the ability of palmitate to inhibit insulin action (Fig. 6A). To determine if mitochondrial transport and β-oxidation is required for effects of palmitate, we treated cells with

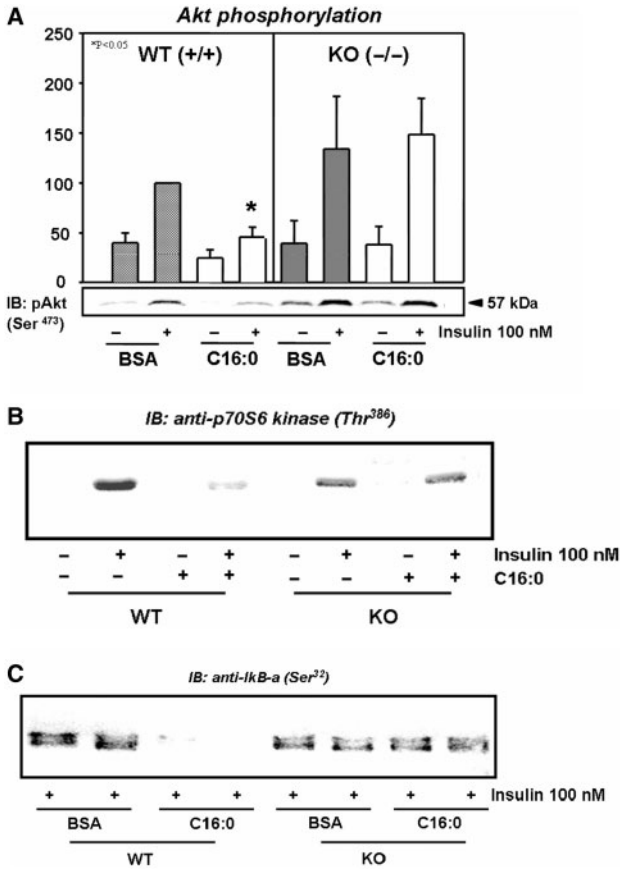

Fig. 5. **Palmitate does not inhibit insulin action in hepatocytes derived from IR-deficient (−/−) mice.** Wild-type (+/+) and IR-deficient (−/−) hepatocytes were differentiated, as described in methods, incubated with BSA or palmitate 250 μM for 12 h, and stimulated with insulin as indicated. Lysates were immunoblotted with anti-phosphoAkt, anti-phospho-p70S6 kinase or anti-phosphoIκB-α. (A) Representative blot and quantification of three experiments are provided for anti-phosphoAkt (A). $^{*}P < 0.05$ for palmitate versus BSA in wild-type cells. (B and C) Representative blots are shown for p70S6 kinase (B) and IκB-α (C).

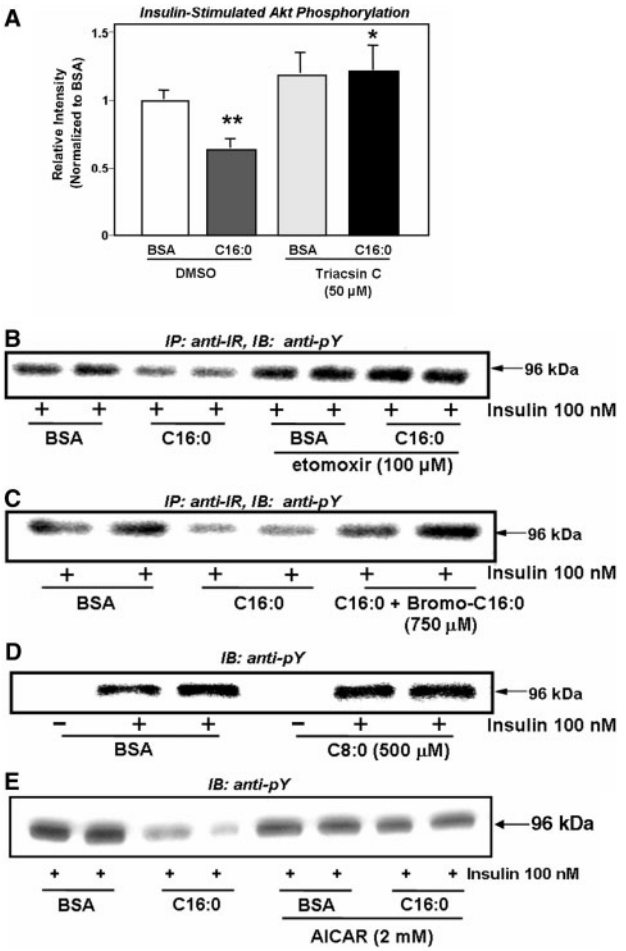

Fig. 6. **Palmitate effects are reversed by triacsin C, etomoxir and AICAR.** (A) Fao cells were incubated with 50 μM triacsin C or DMSO concurrently with either BSA or palmitate 250 μM for 12 h; lysates were immunoblotted with anti-phosphoAkt. $^{*}P = 0.02$ for comparison of palmitate–DMSO versus palmitate–triacsin C; $^{**}P = 0.005$ for palmitate–DMSO versus BSA–DMSO alone. (B–D) Cells were pretreated with BSA or palmitate (250 μM) alone or in combination with (±)-etomoxir (100 μM), bromopalmitate (750 μM), AICAR (2 mM) or C8:0 (500 μM). Following insulin stimulation (100 nM, 30 min), cells were lysed; lysates were immunoprecipitated with anti-IR antibody or directly immunoblotted with anti-phosphotyrosine (4G10) antibody. Representative immunoblots are shown for effects of etomoxir (B), bromopalmitate (C), octanoate (D) and AICAR (E).

(±)-etomoxir (100 μM), a competitive inhibitor of carnitine palmityl transferase (CPT-1) (*33*). Etomoxir was strikingly effective in reversing the effects of palmitate to inhibit IR (Fig. 6B) and Akt phosphorylation, and expression of the IR. Similarly, bromopalmitate, a non-metabolized FA, had no effect on insulin signalling in concentrations up to 750 μM and was unable to reverse the palmitate effects in equimolar concentrations (data not shown). However, in molar excess (a concentration associated with CPT inhibition), bromopalmitate antagonized the effect of palmitate on IR phosphorylation (Fig. 6C). In contrast, octanoate (C8:0, 250 or 500 μM), a medium chain FA that does not require the CPT1 transport system for mitochondrial entry and β-oxidation, had no effect on IR (Fig. 6D) or Akt phosphorylation. Although these data suggest that products of long-chain CoA β-oxidation may contribute to FA-induced cellular effects, perhaps via oxidant stress, we were unable to reverse palmitate effects using the anti-oxidant *N*-acetyl-L-cysteine (1 mM).

Given the apparent requirements of β-oxidation to mediate palmitate effects, we determined whether activation of AMP kinase, a key enzyme inhibiting lipogenesis and increasing lipid oxidative metabolism, might reverse palmitate effects. Treatment with the AMP kinase activator AICAR (2 mM overnight) increased basal levels of IR and Akt phosphorylation and partially reversed the inhibitory effects of palmitate (Fig. 6E).

DISCUSSION

Our study demonstrates that palmitate, a representative abundant saturated FA (*34*), induces insulin resistance at the level of both insulin signalling and gene

expression in two cultured hepatocyte cell lines. These effects are both dose-dependent (low physiologic, 100–750 μM range) and time-dependent. Palmitate inhibits IR and IRS-1/2 tyrosine phosphorylation and activation of PI 3-kinase and Akt. These effects of palmitate extend to inhibition of phosphorylation of p70 S6 kinase, GSK-3 and FOXO1A, likely contributing to reduced insulin-mediated transcriptional changes, and are associated with increased glucose production. It is particularly interesting that FA effects were specific to PI 3-kinase-dependent pathways, as similar selective insulin resistance affecting PI 3-kinase, but not MAP kinase, pathways has been demonstrated in human muscle and endothelial cells (*25*). A strong candidate for the effects of FAs on insulin action is increased diacylglycerol synthesis, leading to activation of novel PKC isoforms θ and ε, in skeletal muscle and liver, respectively (*27*, *35*). PKC activation may increase serine/threonine phosphorylation of IRS-1, reducing its (stimulatory) tyrosine phosphorylation. As expected, we found that palmitate activated phosphorylation and/or translocation of novel PKC isoforms δ and ε. Future work will investigate the levels of DAG generated in response to FA metabolism. In contrast to the reversal of dietary insulin resistance by diabetic arteriosclerosis obliterans (ASO) inhibition of PKCε in rodents with hepatic lipid excess (*36*), we were unable to reverse the effects of palmitate on insulin action with bisindolylmaleimide. IKK-β has been recently implicated as a potential mediator of lipid-induced insulin resistance. However, in Fao cells, neither aspirin nor SN50, an inhibitor of NF-κB nuclear translocation, altered the FA effects. While previous data have also implicated the hexosamine pathway in mediating FA-induced insulin resistance in muscle (*37*), palmitate did not increase levels of UDP-GlcNAc in hepatoma cells, and inhibition of GFAT, the rate limiting enzyme of this pathway, did not reverse palmitate effects. Likewise, specific inhibitors of mitogen-activated protein kinase (MEK), Tor and PI 3-kinase were also unable to reverse the effects of palmitate. Our data indicate that palmitate-induced reduction in IR tyrosine phosphorylation appears to be accounted for by reduced IR mRNA and protein expression. Since the magnitude of the reduction in Akt activation is slightly greater than that of upstream signaling events, we cannot exclude an additional inhibitory effect of FAs at the level of PDK or Akt activation. Furthermore, incubation of IR (−/−) cells with palmitate did not attenuate insulin action at any level. Taken together, these data suggest several hypotheses: (i) modulation of IR mRNA expression or stability is a primary mechanism by which palmitate inhibits insulin action; (ii) since IGF-1 receptors cannot mediate phosphorylation of FOXO1 threonine ^{24} in $IR^{-/-}$ hepatocytes (*38*), phosphorylation of FOXO1 on threonine $^{24/32}$ by kinases downstream of the insulin receptor (not Akt) may be required to observe the inhibitory effects of palmitate on insulin action, particularly at a transcriptional level; and (iii) Since $IR^{-/-}$ hepatocytes can mediate IRS-2 phosphorylation, via the IGF-1 receptor (*19*, *32*) palmitate may exert more specific effects on insulin receptor and *IRS-1-dependent* transcriptional pathways mediating insulin's metabolic effects (*15*). By what mechanism does palmitate reduce insulin receptor mRNA and protein expression? Inhibition of palmitoyl CoA production by triacsin C completely abolishes the effects of palmitate on insulin action, indicating that palmitoyl CoA is the active species. Potential mechanisms for effects of acyl CoA species include: (i) direct modulation of FA-sensitive signal transduction upstream of transcription, resulting in covalent modification of transcription factors, e.g. phosphorylation of FOXO1A or GSK3, or palmitoylation (*39*), (ii) effects on cellular function via metabolism, including oxidation, ATP generation, peroxidation, or lipid metabolites (*10*), (iii) effects at the level of gene transcription, via ligand binding to transcription factors, as demonstrated in some cases for HNF4α (*40*), or alterations in mRNA stability, or (iv) FA regulation of receptor gene expression. For example, palmitoyl CoA is an important precursor of ceramide, an intermediate that can induce resistance to insulin and other growth factors at the level of Akt membrane localization and phosphorylation (*41*). However, since etomoxir increases ceramide synthesis and palmitate-induced apoptosis (*42*), yet blocks the effect of palmitate in our model, it is unlikely that ceramide synthesis contributes to FA-induced effects in hepatic cells. Future work will investigate whether the levels of ceramides are augmented in response to FA treatments in this model. The ability of etomoxir to inhibit palmitate effects supports a role for mitochondrial transport and/or β-oxidation. Conversely, the AMP kinase activator AICAR, which stimulates β-oxidation, reverses palmitate effects. While reactive oxygen species (ROS) have been recently linked to certain forms of insulin resistance, we find that anti-oxidant therapy cannot reverse palmitate-induced insulin resistance in either hepatoma cells or C2C12 myotubes (*43*). Thus our data are more consistent with the model indicating that products of incomplete β-oxidation can mediate insulin resistance (*44*, *45*). Our data are also in accord with previous studies demonstrating that FFA inhibition of insulin binding does not occur in hepatocytes pre-treated with etomoxir, methylpalmoxirate (another CPT-1 inhibitor) or potassium cyanide, an electron transport chain inhibitor (*33*). Moreover, liver-specific inhibition of CPT-1 by SDZ-CPI-975 lowers glucose in rats and primates (*46*). It is surprising, however, that high doses of octanoate, a medium chain FA that does not require CPT-1 for mitochondrial transport but can be oxidized, does not inhibit insulin action. Finally, both polyunsaturated and saturated FAs, including palmitate, can alter key regulatory transcription factors (e.g. PGC1β) (*43*) and post-transcriptional regulation of mRNA stability (*47*). FAs and fatty acyl CoA can serve as direct ligands for transcription factors, including PPAR and HNF families, respectively (*40*, *48*). Direct binding of palmitate to PPAR α, γ or δ would be an unlikely mechanism, since acyl CoA synthesis is required, and binding of palmitoyl CoA to PPAR is limited (*49*). Moreover, although PPARα is abundant in liver and can bind to palmitate (IC_{50} of 1.5 μM) and other long-chain saturated FAs, the PPARα agonists WY14643 or EYTA were unable to mediate the effects of palmitate in our model (data not shown). A direct effect of palmitoyl CoA on transcription would seem less likely, since etomoxir reversed the effects of palmitate. Alternatively, we cannot exclude that the inhibitory effect of etomoxir

and high-dose bromopalmitate might be related to direct competitive binding of etomoxir CoA to nuclear receptors or displacement of palmitate binding by bromopalmitate. Similarly, an alternative explanation for the C8 data is that medium-chain saturated FAs have limited affinity for nuclear receptors, as has been shown for PPAR (*48*). However, taken together, these data suggest that a direct effect of palmitoyl CoA on transcription is less likely and again point to a role for products of β-oxidation to alter insulin action and gene transcription (*34*). In a broader physiologic perspective, our studies provide a potential mechanism for observations that saturated FAs and central obesity are associated with reduced insulin clearance by the liver. After just 6 weeks of high-fat feeding, insulin clearance is reduced by 50% in dogs, suggesting a reduction in hepatocyte IR number and/or function, perhaps mediated by portal FA flux. These data are potentially relevant for the pathogenesis of DM, as decreased hepatic insulin receptor expression can contribute to hepatic insulin resistance and reduced insulin clearance, thus aggravating peripheral hyperinsulinaemia and insulin resistance (*16*). Taken together, our data provide direct evidence that a representative saturated FA, sodium palmitate, inhibits the metabolic actions of insulin and attenuates insulin signal transduction in two well-differentiated hepatocyte cell lines. Moreover, we demonstrate that the inhibition of insulin signalling is mediated at the level of IR expression and requires generation of the CoA species. Further work will be directed at defining the precise molecular mechanism(s) by which palmitoyl CoA or its oxidation product(s) inhibit insulin action and modulate IR expression.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Boden, G. (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46**, 3–10
2. Rebrin, K., Steil, G.M., Getty, L., and Bergman, R.N. (1995) Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* **44**, 1038–1045
3. Sindelar, D.K., Chu, C.A., Rohlie, M., Neal, D.W., Swift, L.L., and Cherrington, A.D. (1997) The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes* **46**, 187–196
4. Randle, P.J., Garland, P.B., Hales, C.N., and Newsholme, F.A. (1963) The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1**, 785–789
5. Roden, M., Krssak, M., Stingl, H., Gruber, S., Hofer, A., Furnsinn, C., Moser, E., and Waldhausl, W. (1999) Rapid impairment of skeletal muscle glucose transport/phosphorylation by free fatty acids in humans. *Diabetes* **48**, 358–364
6. Dresner, A., Laurent, D., Marcucci, M., Griffin, M.E., Dufour, S., Cline, G.W., Slezak, L.A., Andersen, D.K., Hundal, R.S., Rothman, D.L., Petersen, K.F., and Shulman, G.I. (1999) Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J. Clin. Invest.* **103**, 253–259
7. Richardson, D.K., Kashyap, S., Bajaj, M., Cusi, K., Mandarino, S.J., Finlayson, J., DeFronzo, R.A., Jenkinson, C.P., and Mandarino, L.J. (2004) Lipid infusion decreases the expression of nuclear encoded mitochondrial genes and increases expression of extracellular matrix genes in human skeletal muscle. *J. Biol. Chem*. **280**, 10290–10297
8. Muoio, D.M. and Newgard, C.B. (2006) Obesity-related derangements in metabolic regulation. *Annu. Rev. Biochem.* **75**, 367–401
9. Boden, G., She, P., Mozzoli, M., Cheung, P., Gumireddy, K., Reddy, P., Xiang, X., Luo, Z., and Ruderman, N. (2005) Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. *Diabetes* **54**, 3458–3465
10. Holland, W.L., Brozinick, J.T., Wang, L.P., Hawkins, E.D., Sargent, K.M., Liu, Y., Narra, K., Hoehn, K.L., Knotts, T.A., Siesky, A., Nelson, D.H., Karathanasis, S.K., Fontenot, G.K., Birnbaum, M.J., and Summers, S.A. (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* **5**, 167–179
11. Itani, S.I., Ruderman, N.B., Schmieder, F., and Boden, G. (2002) Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* **51**, 2005–2011
12. Obici, S., Wang, J., Chowdury, R., Feng, Z., Siddhanta, U., Morgan, K., and Rossetti, L. (2002) Identification of a biochemical link between energy intake and energy expenditure. *J. Clin. Invest.* **109**, 1599–1605
13. Michael, M.D., Kulkarni, R.N., Postic, C., Previs, S.F., Shulman, G.I., Magnuson, M.A., and Kahn, C.R. (2000) Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol. Cell* **6**, 87–97
14. Withers, D.J., Gutierrez, J.S., Towery, H., Burks, D.J., Ren, J.M., Previs, S., Zhang, Y., Bernal, D., Pons, S., Shulman, G.I., Bonner-Weir, S., and White, M.F. (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391**, 900–904
15. Taniguchi, C.M., Ueki, K., and Kahn, R. (2005) Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. *J. Clin. Invest.* **115**, 718–727
16. Seppala-Lindroos, A., Vehkavaara, S., Hakkinen, A.M., Goto, T., Westerbacka, J., Sovijarvi, A., Halavaara, J., and Yki-Jarvinen, H. (2002) Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J. Clin. Endocrinol. Metab.* **87**, 3023–3028
17. Rother, K.I., Imai, Y., Caruso, M., Beguinot, F., Formisano, P., and Accili, D. (1998) Evidence that IRS-2 phosphorylation is required for insulin action in hepatocytes. *J. Biol. Chem.* **273**, 17491–17497
18. Cheatham, B., Vlahos, C.J., Cheatham, L., Wang, L., Blenis, J., and Kahn, C.R. (1994) Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. *Mol. Cell. Biol.* **14**, 4902–4911
19. Kriauciunas, K.M., Goldstein, B.J., Lipes, M.A., and Kahn, C.R. (1993) Modulation of expression of insulin and IGF-I receptor by Epstein-Barr virus and its gene products LMP and EBNA-2 in lymphocyte cell lines. *J. Cell. Physiol.* **154**, 486–495

20. Hebert, L.F. Jr., Daniels, M.C., Zhou, J., Crook, E.D., Turner, R.K., Simmons, S.T., Neidigh, J.L., Zhu, J.S., Baron, A.D., and McClain, D.A. (1996) Overexpression of glutamine: fructose-6-phosphate amidotransferase in transgenic mice leads to insulin resistance. *J. Clin. Invest.* **98**, 930–936
21. Cho, H., Mu, J., Kim, J.K., Thorvaldsen, J.L., Chu, Q., Crenshaw, E.B. III., Kaestner, K.H., Bartolomei, M.S., Shulman, G.I., and Birnbaum, M.J. (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science* **292**, 1728–1731
22. Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M., and Hemmings, B.A. (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378**, 785–789
23. Naimi, M., Gautier, N., Chaussade, C., Valverde, A.M., Accili, D., and Van Obberghen, E. (2007) Nuclear forkhead box O1 controls and integrates key signaling pathways in hepatocytes. *Endocrinology* **148**, 2424–2434
24. Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M.E., Mandel, G., DeFronzo, R.A., Kahn, C.R., and Mandarino, L.J. (2000) Insulin resistance differentially affects the PI 3-kinase-and MAP kinase - mediated signaling in human muscle. *J. Clin. Invest.* **105**, 311–320
25. Jiang, Z.Y., Lin, Y.W., Clemont, A., Feener, E.P., Hein, K.D., Igarashi, M., Yamauchi, T., White, M.F., and King, G.L. (1999) Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. *J. Clin. Invest.* **104**, 447–457
26. Wolfrum, C., Borchers, T., Sacchettini, J.C., and Spener, F. (2000) Binding of fatty acids and peroxisome proliferators to orthologous fatty acid binding proteins from human, murine, and bovine liver. *Biochemistry* **39**, 1469–1474
27. Griffin, M.E., Marcucci, M.J., Cline, G.W., Bell, K., Barucci, N., Lee, D., Goodyear, L.J., Kraegen, E.W., White, M.F., and Shulman, G.I. (1999) Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* **48**, 1270–1274
28. Kim, J.K., Kim, Y.J., Fillmore, J.J., Chen, Y., Moore, I., Lee, J., Yuan, M., Li, Z.W., Karin, M., Perret, P., Shoelson, S.E., and Shulman, G.I. (2001) Prevention of fat-induced insulin resistance by salicylate. *J. Clin. Invest.* **108**, 437–446
29. Hennes, M.M., Shrago, E., and Kissebah, A.H. (1990) Receptor and postreceptor effects of free fatty acids (FFA) on hepatocyte insulin dynamics. *Int. J. Obes.* **14**, 831–841
30. Usui, I., Takata, Y., Imamura, T., Morioka, H., Sasaoka, T., Sawa, T., Ishihara, H., Ishiki, M., and Kobayashi, M. (1997) Fatty acid induced insulin resistance in rat-1 fibroblasts overexpressing human insulin receptors: impaired insulin-stimulated mitogen-activated protein kinase activity. *Diabetologia* **40**, 894–901
31. Schmitz-Peiffer, C., Browne, C.L., Oakes, N.D., Watkinson, A., Chisholm, D.J., Kraegen, E.W., and Biden, T.J. (1997) Alterations in the expression and cellular localization of protein kinase C isozymes epsilon and theta are associated with insulin resistance in skeletal muscle of the high-fat-fed rat. *Diabetes* **46**, 169–178
32. Park, B., Kido, Y., and Accili, D. (1999) Differential signaling of insulin and IGF-1 receptors to glycogen synthesis in murine hepatocyes. *Biochemistry* **38**, 7517–7523
33. Spurway, T.D., Pogson, C.I., Sherratt, H.S., and Agius, L. (1997) (404) Etomoxir, sodium 2-[6-(4-chlorophenoxy)hexyl] oxirane-2-carboxylate, inhibits triacylglycerol depletion in hepatocytes and lipolysis in adipocytes. *FEBS Lett*. **404**, 111–114
34. Dey, D., Bhattacharya, A., Roy, S., and Bhattacharya, S. (2007) Fatty acid represses insulin receptor gene expression by impairing HMGA1 through protein kinase Cε. *Biochem. Biophys. Res. Commun.* **357**, 474–479
35. Bell, K., Schmitz-Peiffer, C., Lim-Fraser, M., Biden, T.J., Cooney, G.J., and Kraegen, E.W. (2000) Acute reversal of lipid-induced muscle insulin resistance is associated with rapid alteration in PKC-theta localization. *Am. J. Physiol. Endocrinol. Metab.* **279**, 1196–1201
36. Samuel, V.T., Liu, Z.X., Wang, A., Beddow, S.A., Geisler, J.G., Kahn, M., Zhang, X.M., Monia, B.P., Bhanot, S., and Shulman, G.I. (2007) Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. *J. Clin. Invest.* **117**, 739–745
37. Hawkins, M., Barzilai, N., Liu, R., Hu, M., Chen, W., and Rossetti, L. (1997) Role of the glucosamine pathway in fat-induced insulin resistance. *J. Clin. Invest.* **99**, 2173–2182
38. Nakae, J., Barr, V., and Accili, D. (2000) Differential regulation of gene expression by insulin and IGF-1 receptors correlates with phosphorylation of a single amino acid residue in the forkhead transcription factor FKHR. *EMBO J.* **19**, 989–996
39. Webb, Y., Hermida-Matsumoto, L., and Resh, M.D. (2000) Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. *J. Biol. Chem.* **275**, 261–270
40. Hertz, R., Magenheim, J., Berman, I., and Bar-Tana, J. (1998) (392) Fatty acyl-CoA thioesters are ligands of hepatic nuclear factor-4alpha. *Nature* **392**, 512–516
41. Schmitz-Peiffer, C., Craig, D.L., and Biden, T.J. (1999) Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. *J. Biol. Chem.* **274**, 24202–24210
42. Paumen, M.B., Ishida, Y., Muramatsu, M., Yamamoto, M., and Honjo, T. (1997) Inhibition of carnitine palmitoyltransferase I augments sphingolipid synthesis and palmitate-induced apoptosis. *J. Biol. Chem.* **272**, 3324–3329
43. Crunkhorn, S., Dearie, F., Mantzoros, C., Gami, H., da Silva, W.S., Espinoza, D., Faucette, R., Barry, K., Bianco, A.C., and Patti, M.E. (2007) PGC-1 expression is reduced in obesity: potential pathogenic role of saturated fatty acids and p38 map kinase activation. *J. Biol. Chem*. **282**, 15439–15450
44. Koves, T.R., Li, P., An, J., Akimoto, T., Slentz, D., Ilkayeva, O., Dohm, G.L., Yan, Z., Newgard, C.B., and Muoio, D.M. (2005) Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. *J. Biol. Chem.* **280**, 33588–33598
45. Koves, T.R., Ussher, J.R., Noland, R.C., Sientz, D., Mosedale, M., Ilkayeva, O., Bain, J., Stevens, R., Dyck, J.R.B., Newgard, C.B., Lopaschuk, G.D., & Muoio, D.M. (2008) Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab*. **7**, 45–56
46. Deems, R.O., Anderson, R.C., and Foley, J.E. (1998) (274) Hypoglycemic effects of a novel fatty acid oxidation inhibitor in rats and monkeys. *Am. J. Physiol.* **274**, R524–R528
47. Duplus, E., Glorian, M., and Forest, C. (2000) Fatty acid regulation of gene transcription. *J. Biol. Chem.* **275**, 30749–30752
48. Xu, H.E., Lambert, M.H., Montana, V.G., Parks, D.J., Blanchard, S.G., Brown, P.J., Sternbach, D.D., Lehmann, J.M., Wisely, G.B., Willson, T.M., Kliewer, S.A., and Milburn, M.V. (1999) Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell* **3**, 397–403
49. Desvergne, B. and Wahli, W. (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20**, 649–688