Phosphorylation of Adaptor Protein Containing Pleckstrin Homology Domain, Phosphotyrosine Binding Domain, and Leucine Zipper Motif 1 (APPL1) at Ser\(^{430}\) Mediates Endoplasmic Reticulum (ER) Stress-induced Insulin Resistance in Hepatocytes*

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**Significance:** PKC\(_{\alpha}\)-mediated APPL1 phosphorylation could be a potential drug target for the treatment of insulin resistance.

**Background:** Adaptor protein APPL1 plays a critical role in regulating both adiponectin and insulin signaling pathways. Results: ER stress-induced APPL1 phosphorylation at Ser\(^{430}\) blocks the insulin-sensitizing effect of APPL1 in a PKC\(_{\alpha}\)-dependent manner. Conclusion: APPL1 phosphorylation at Ser\(^{430}\) mediates ER stress-induced insulin resistance.

APPL1 is an adaptor protein that plays a critical role in regulating adiponectin and insulin signaling. However, how APPL1 is regulated under normal and pathological conditions remains largely unknown. In this study, we show that APPL1 undergoes phosphorylation at Ser\(^{430}\) and that this phosphorylation is enhanced in the liver of obese mice displaying insulin resistance. In cultured mouse hepatocytes, APPL1 phosphorylation at Ser\(^{430}\) is stimulated by phorbol 12-myristate 13-acetate, an activator of classic PKC isoforms, and by the endoplasmic reticulum (ER) stress inducer, thapsigargin. Overexpression of wild-type but not dominant negative PKC\(_{\alpha}\) increases APPL1 phosphorylation at Ser\(^{430}\) in mouse hepatocytes. In addition, suppressing PKC\(_{\alpha}\) expression by shRNA in hepatocytes reduces ER stress-induced APPL1 phosphorylation at Ser\(^{430}\) as well as the inhibitory effect of ER stress on insulin-stimulated Akt phosphorylation. Consistent with a negative regulatory role of APPL1 phosphorylation at Ser\(^{430}\) in insulin signaling, overexpression of APPL1\(^{S430D}\) but not APPL1\(^{S430A}\) impairs the potentiating effect of APPL1 on insulin-stimulated Akt phosphorylation at Thr\(^{308}\). Taken together, our results identify APPL1 as a novel target in ER stress-induced insulin resistance and PKC\(_{\alpha}\) as the kinasemediating ER stress-induced phosphorylation of APPL1 at Ser\(^{430}\).

Adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif 1 (APPL1) is the first adiponectin receptor-binding protein identified to play a critical role in regulating adiponectin signaling including activation of AMPK,3 p38 MAPK, and endothelial NOS pathways in muscle and endothelial cells (1–5). In addition to binding to adiponectin receptors, APPL1 has been found to interact with the catalytic subunit of phosphatidylinositol 3-kinase (p110) and Akt (6) and to control the substrate specificity of Akt toward GSK-3\(_{\beta}\) but not TSC2 (7). The association of APPL1 and Akt appears to play a positive role in insulin signaling in liver tissue by blocking the association of Akt with its endogenous inhibitor tribble 3 (TRB3), thus promoting Akt translocation to the plasma membrane and the endosomes for further activation (8). Consistent with this, insulin-stimulated Akt activation and GLUT4 translocation were significantly reduced in APPL1-suppressed C2C12 myocytes and 3T3-L1 adipocytes (2, 9).

The precise mechanisms by which APPL1 mediates adiponectin and insulin signaling remain largely unknown. Available evidence reveals that APPL1 contains multiple potential phosphorylation sites (Ser\(^{151}\), Ser\(^{401}\), Ser\(^{427}\), Ser\(^{430}\), and Ser\(^{691/693/696}\)) (10). However, the roles of the phosphorylation and the upstream kinases of APPL1 in regulating insulin sensitivity are unknown. In the current study, we demonstrate that APPL1 undergoes ER stress-induced and PKC\(_{\alpha}\)-dependent phosphorylation at Ser\(^{430}\) in hepatocytes. In addition, phosphorylation at Ser\(^{430}\) impairs the insulin-sensitizing effect of APPL1 in hepatocytes. Our study reveals a novel mechanism by which ER stress induces insulin resistance and demonstrates...
that PKCα is a direct kinase for APPL1 phosphorylation, making PKCα a potential drug target for the treatment of obesity and its related diseases.

**MATERIALS AND METHODS**

**Plasmids, Chemicals, and Antibodies**—The plasmids encoding full-length human APPL1 were described previously (2). The plasmids encoding S430A and S430D mutated APPL1 were generated by site-directed mutagenesis from wild-type APPL1 plasmid. The RNAi-resistant APPL1 constructs (wild-type APPL1, S430A, and S430D plasmids) were generated by site-directed mutagenesis with a primer (5′-GTG GAT ATG CAC AAT AAA C-3′) that targets the APPL1 cDNA nucleotide sequence from 1154 to 1173 to abolish the RNAi target nucleotide sequence without changing the encoded protein sequence. Plasmids encoding wild-type and dominant negative PKCα were kindly provided by Dr. Jae-Won Soh (11). The PKCα scramble and shRNA constructs were from Santa Cruz Biotechnology (Santa Cruz, CA).

The following compounds were used in our studies. Gö 6976 and Gö 6983 were purchased from Calbiochem. Thapsigargin, recombinant PKC enzyme kits, PMA, AICAR, and H2O2 were from Sigma.

An antibody to APPL1 was described previously (2). The phosphor-antibody against APPL1 was generated by 21st Century Biochemicals (Marlboro, MA). Antibodies against AMPKα, phosphor-AMPKα-Thr172, Akt, phosphor-Akt-Thr308, ERK1/2, phosphor-ERK1/2-Thr202/Tyr204, phosphor-elF2α-Ser2, elF2α, and β-actin were purchased from Cell Signaling Technology (Danvers, MA). An antibody against the HA tag was purchased from Covance (Emeryville, CA). The monoclonal anti-Myc antibody was generated in-house as described before (4). The anti-β-tubulin 2.1 antibody was purchased from Sigma.

**Transfection of Hepatocytes**—DNA constructs were introduced into mouse hepatocytes by electroporation using the Neon transfection system (Invitrogen). In brief, HepIR cells were serum-starved for 4 h followed with chemical treatment. —Mouse hepatocytes were transfected with cDNA encoding Myc-tagged APPL1. Thirty-six hours after transfection, the cells were washed with PBS and suspended in R buffer from the same company. Cell mixtures (100 µl) containing about 10⁶ cells and 5 μg of DNA were loaded into a particular tip. Voltage was adjusted to 1,230 V, and pulse length was adjusted to 20 ms (two pulses). Thirty-six hours after transfection, the cells were serum-starved for 4 h followed with chemical treatment.

**In Vivo Labeling**—Mouse hepatocytes were transfected with cDNA encoding Myc-tagged APPL1. Thirty-six hours after transfection, cells were in vivo labeled with [32P]orthophosphate according to a protocol described previously (12) and treated with (+) or without (−) 2 µM PMA for 30 min. APPL1 was immunoprecipitated with an antibody to the Myc tag, and APPL1 phosphorylation was visualized by autoradiography and quantified by using a PhosphorImager. The expression of APPL1 in these cells was confirmed by Western blot analysis using the anti-Myc antibody.

**In Vitro Phosphorylation of APPL1 by PKCα or Other PKC Isoforms**—GST-fused APPL1 was incubated with different recombinant PKC isoforms to determine the in vitro phosphorylation of APPL1 as described previously (12). Wild type and S430A mutant of APPL1 were purified by immunoprecipitation from C2C12 cells transiently expressing these proteins. Immunoprecipitated APPL1 proteins were incubated in 30 µl of kinase assay buffer (50 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 1 mM Na₃VO₄, 1 mM sodium pyrophosphate, 1 mM NaF, and 1 mM phenylmethylsulfonyl fluoride) containing purified PKCα and 2 µCi of [γ-32P]ATP. In vitro phosphorylation reaction was carried out for 30 min at 30 °C, and in vitro phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography.

**Animal Studies**—Male db/db mice and their lean controls (stock number 000642, 3–5 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME). The mice were group-housed in a specific pathogen-free facility at 22–24 °C on a 12-h light/12-h dark cycle with the lights on at 8:00 a.m. The mice were fed with standard rodent chow, and all animals had access to water ad libitum. At 12 weeks of age, mice were then sacrificed, and the tissues were isolated according to the procedure described in our recent studies (13). For high fat diet experiments, 5-week-old C57BL/6 male mice (10 mice per group) were fed with normal chow diet (Research Diets number D12328: 10.5 kcal% fat, 73.1 kcal% carbohydrate, and 16.4 kcal% protein) or high fat diet (Research Diets number D12330: 58.0 kcal% fat, 16.0 kcal% protein, and 26 kcal% carbohydrate) for 5 months. Mice were sacrificed, and the tissues were isolated as described (13). All animal procedures were approved by the University of Texas Health Science Center Animal Care and Use Committee.

**Statistical Analysis**—Quantification of APPL1 phosphorylation at Ser430 or Akt phosphorylation at Thr308 was performed by Western blot analysis using the National Institutes of Health Image software and was normalized with the amount of protein expression of APPL1 or Akt, respectively, in each experiment. Results were expressed as the mean ± S.E. Differences between the groups were examined for statistical significance using analysis of variance. * indicates p < 0.05; ** indicates p < 0.01.

**RESULTS**

**Identification of Ser430 as a PMA-stimulated APPL1 Phosphorylation Site**—APPL1 is involved in multiple intracellular cell signaling events including adiponectin and insulin signaling. However, how the protein is regulated in cells remains elusive. A recent mass spectrometry study revealed that APPL1 is phosphorylated at several sites including Ser151, Ser401, Ser427, Ser430, and Ser691/693/696 and that the protein could be a potential target for multiple important cellular kinases including PKC, AMPK, and MAPK family members Erk, p38 MAPK, and c-Jun N-terminal kinase (JNK) (10). To determine whether these kinases were involved in the phosphorylation of APPL1, we performed in vivo labeling experiments with C2C12 cells transiently expressing Myc-APPL1. Treating the labeled cells with the PKC activator PMA but not the AMPK activator AICAR or the MAPK activator H2O2 (14) led to a marked increase in APPL1 phosphorylation (Fig. 1A), suggesting that PKC, but not AMPK and MAPK, is involved in the phosphorylation of APPL1.

To map the region of APPL1 involved in PMA-stimulated phosphorylation, C2C12 cells expressing Myc-tagged full-
length or truncated forms of APPL1 were in vivo labeled with $[^{32}P] \text{orthophosphate}$ and treated with or without PMA. We found that APPL1 (1–471) but not APPL1 (1–269) was phosphorylated in the cells in response to PMA treatment (Fig. 1B), suggesting that PMA stimulates phosphorylation of APPL1 in a region containing amino acids from 269 to 471. This region contains the pleckstrin homology (PH) domain and a fragment between the pleckstrin homology domain and the phosphotyrosine binding (PTB) domain, which contains three putative phosphorylation sites, Ser401, Ser427, and Ser430 (10). Western blot analysis using the phosphospecific antibody to Ser430 indicated that PMA stimulated the phosphorylation of endogenous APPL1 at Ser430 in hepatocytes (Fig. 1C), which is consistent with a recent study showing that APPL1 is phosphorylated at Ser430 in HEK293 cells (10).

**Phosphorylation of APPL1 at Ser430 Is Associated with Obesity and Insulin Resistance**—To determine the physiological relevance of APPL1 phosphorylation at Ser430, we examined APPL1 phosphorylation in the liver of db/db mice. As shown in Fig. 2, A and B, APPL1 Ser430 phosphorylation was dramatically elevated in the liver tissues of db/db mice as compared with those of lean control mice, suggesting that phosphorylation of APPL1 at Ser430 is associated with obesity and insulin resistance. Consistent with this finding, APPL1 phosphorylation at Ser430 was also enhanced in the liver tissues of HFD-fed mice (Fig. 2C and D).

**APPL1 Phosphorylation at Ser430 Is Mediated by PKC**—The finding that APPL1 phosphorylation at Ser430 is stimulated by PMA suggests that a classic PKC isoform is involved in the phosphorylation. To identify the PKC isoform involved in the phosphorylation of APPL1 at Ser430, different recombinant PKC isoforms were incubated with purified GST-APPL1 fusion protein in the presence of $[\gamma-^{32}P] \text{ATP}$. The in vitro kinase assays showed that incubation with PKCα, but not PKCβ1 and
ER Stress Induces APPL1 Phosphorylation at Ser\(^{430}\)

**FIGURE 3.** Phosphorylation of APPL1 at Ser\(^{430}\) is mediated by PKC\(\alpha\) in hepatocytes. A, the phosphorylation of APPL1 by PKC\(\alpha\) in vitro. GST-fused APPL1 was purified from bacteria and phosphorylated in vitro in the presence of PKC\(\alpha\) and recombinant PKC isoforms. The phosphorylation of APPL1 was visualized by autoradiography ( Autorad ), and phosphorylated APPL1 at Ser\(^{430}\) was detected by Western blot analysis with specific antibodies as indicated. B, PKC\(\alpha\)-stimulated phosphorylation of APPL1 at Ser\(^{430}\) was significantly reduced in PKC\(\alpha\)-suppressed hepatocytes, further demonstrating a negative regulatory role of PKC\(\alpha\) in mediating ER stress-induced phosphorylation of APPL1 at Ser\(^{430}\). Consistent with this, TG-stimulated phosphorylation of APPL1 at Ser\(^{430}\) was reduced in PKC\(\alpha\)-suppressed hepatocytes, further indicating an important role of PKC\(\alpha\) in mediating ER stress-induced insulin resistance in hepatocytes.

**DISCUSSION**

APPL1, the first signaling molecule identified downstream of adiponectin receptors, has been shown to play an important role in adiponectin signaling and action (2–5, 17, 19). In addition to binding to adiponectin receptors, APPL1 has also been shown to interact with PI3K and Akt (6) and facilitate Akt activation and substrate specificity (7). APPL1 potentiates insulin-stimulated Akt activation and subsequent suppression of hepatic glucose production through counteracting the inhibition of Akt activation by TRB3 in the liver (8). However, how APPL1 is regulated in cells remains largely unknown. A recent
study predicted that APPL1 could be phosphorylated at multiple residues, suggesting that post-modification could be a potential mechanism regulating APPL1 action in cells (10). However, the kinases that mediate APPL1 phosphorylation and the functional roles of the phosphorylation are unknown. In the present study, we demonstrate that APPL1 undergoes phosphorylation at Ser430 under obesity and ER stress conditions. In addition, we demonstrate that phosphorylation at this site impairs the insulin-sensitizing effect of APPL1. Furthermore, we have identified PKCα as the kinase mediating ER stress-induced phosphorylation of APPL1 at Ser430 in hepatocytes. However, how APPL1 phosphorylation at Ser430 negatively regulates the insulin-sensitizing effect of APPL1 remains elusive. Because APPL1 stimulates AMPK and p38 MAPK phosphorylation by interacting with AdipoR1, LKB1, PP2A, PKCα, and Rab5 in myotubes (1–4), it is possible that phosphorylation of APPL1 at Ser430 down-regulates adiponectin signaling by inhibiting APPL1 from binding to AdipoR1, LKB1, PP2A, PKCα, and/or Rab5. It is also possible that phosphorylation of APPL1 at Ser430 affects the association between APPL1 and PI3K or Akt (6, 7). Future studies are needed to investigate the mechanisms.

ER stress has been shown to contribute to obesity-induced insulin resistance (15, 20, 21). ER stress impairs insulin signaling through hyperactivation of JNK and subsequent stimula-
tion of phosphorylation of the insulin receptor substrate-1 (IRS-1) at Ser307 (15). Serine phosphorylation of IRS-1 suppresses its tyrosine phosphorylation and thus the functional roles of this adaptor protein, which contributes to ER stress-induced insulin receptor degradation (16). Our current study indicates that APPL1 phosphorylation at Ser430 is also involved in ER stress-induced insulin resistance.

Activation of PKC isoforms has been shown to be associated with obesity and insulin resistance (22, 23). PKCα, one classic PKC isoform, was found to mediate the suppressing effect of TNFα on insulin action and signaling by promoting IRS-1 serine/threonine phosphorylation (24) and inhibiting IR tyrosine phosphorylation (17, 25). PKCα has also been shown to mediate the inhibitory effect of advanced glycation end products on insulin action in the muscle through the formation of a multifunctional complex including RAGE-IRS-1-Src (18). In our present study, we found that induction of ER stress stimulated the phosphorylation of APPL1 at Ser430 via a PKCα-dependent mechanism. Overexpression of wild-type APPL1 and the S430A mutant, but not the S430D mutant, increased insulin-stimulated Akt phosphorylation. These data further suggest that the phosphorylation of APPL1 at Ser430 reduces the insulin-sensitizing effect of APPL1, which is a novel mechanism underlying ER stress-stimulated insulin resistance. In summary, our study has demonstrated for the first time that APPL1 mediates ER stress-induced and PKCα-mediated phosphorylation at Ser430 and that the phosphorylation negatively regulates APPL1 function in hepatocytes. This result suggests that APPL1 phosphorylation at Ser430 could be an important mechanism underlying obesity- and ER stress-induced insulin resistance.

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REFERENCES

1. Deepa, S. S., Zhou, L., Ryu, J., Wang, C., Mao, X., Li, C., Zhang, N., Musi, N., DeFronzo, R. A., Liu, F., and Dong, L. Q. (2011) APPL1 mediates adiponectin-induced LKB1 cytosolic localization through the PP2A-PKCζ signaling pathway. Mol. Endocrinol. 25, 1773–1785
2. Mao, X., Kikani, C. K., Riojas, R. A., Langlais, P., Wang, L., Ramos, F. J., Fang, Q., Christ-Roberts, C. Y., Hong, J. Y., Kim, R. Y., Liu, F., and Dong, L. Q. (2006) APPL1 binds to adiponectin receptors and mediates adiponectin signaling and function. Nat. Cell Biol. 8, 516–523
3. Xin, X., Zhou, L., Reyes, C. M., Liu, F., and Dong, L. Q. (2011) APPL1 mediates adiponectin-stimulated p38 MAPK activation by scaffolding the TAK1-MK3-p38 MAPK pathway. Am. J. Physiol. Endocrinol. Metab. 300, E103–E110
4. Zhou, L., Deepa, S. S., Etzler, J. C., Ryu, J., Mao, X., Fang, Q., Liu, D. D., Torres, J. M., Jia, W., Lechleiter, J. D., Liu, F., and Dong, L. Q. (2009) Adiponectin activates AMP-activated protein kinase in muscle cells via APPL1/LKB1-dependent and phosphorylase Cα1/Cα2/calcium-dependent protein kinase–catheme-dependent pathways. J. Biol. Chem. 284, 22426–22435
5. Cheng, K. K., Lam, K. S., Wang, Y., Huang, Y., Carling, D., Wu, D., Wong, C., and Xu, A. (2007) Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. Diabetes 56, 1387–1394
6. Mitsuhashi, Y., Johnson, S. W., Sonoda, G., Tanno, S., Golemis, E. A., and Testa, J. R. (1999) Identification of a chromosome 3p14.3–21.1 gene, APPL1, encoding an adaptor molecule that interacts with the oncoprotein-serine/threonine kinase AKT2. Oncogene 18, 4891–4898
7. Schenck, A., Goto-Silva, L., Collinet, C., Rhinn, M., Giner, A., Habermann, B., Brand, M., and Zerial, M. (2008) The endosomal protein APPL1 mediates Akt substrate specificity and cell survival in vertebrate development. Cell 133, 486–497
8. Chen, K. K., Iglesias, M. A., Lam, K. S., Wang, Y., Sweeney, G., Zhu, W., Vanhoutte, P. M., Kraegen, E. W., and Xu, A. (2009) APPL1 potentiates insulin-mediated inhibition of hepatic glucose production and alleviates diabetes via Akt activation in mice. Cell Metab. 9, 417–427
9. Saito, T., Jones, C. C., Huang, S., Czech, M. P., and Pilk, P. F. (2007) The interaction of Akt with APPL1 is required for insulin-stimulated Glut4 translocation. J. Biol. Chem. 282, 32280–32287
10. Gant-Bramall, R. L., Brousard, J. A., Mahsut, A., Webb, D. J., and McLean, J. A. (2010) Identification of phosphorylation sites within the signaling adaptor APPL1 by mass spectrometry. J. Proteome Res. 9, 1541–1548
11. Soh, J. W., and Weinstein, I. B. (2003) Roles of specific isoforms of protein kinase C in the transcriptional control of cyclin D1 and related genes. J. Biol. Chem. 278, 34709–34716
12. Dong, L. Q., Du, H., Porter, S. G., Kolakowski, L. F., Jr., Lee, A. V., Mandarino, L. J., Fan, J., Yee, D., and Liu, F. (1997) Cloning, chromosome localization, expression, and characterization of an Src homology 2 and pleckstrin homology domain-containing insulin receptor-binding protein hGrb10. J. Biol. Chem. 272, 29104–29112
13. Wang, L., Balas, B., Christ-Roberts, C. Y., Kim, R. Y., Ramos, F. J., Kikani, C. K., Li, C., Deng, C., Reyna, S., Musi, N., Dong, L. Q., DeFronzo, R. A., and Liu, F. (2007) Peripheral disruption of the Grb10 gene enhances insulin signaling and sensitivity in vivo. Mol. Cell. Biol. 27, 6497–6505
14. Tournier, C., Thomas, G., Pierre, J., Jacquemin, C., Pierre, M., and Saunier, B. (1997) Mediation by arachidonic acid metabolites of the H2O2-induced stimulation of mitogen-activated protein kinases (extracellular signal-regulated kinase and c-jun NH2-terminal kinase). Eur. J. Biochem. 244, 587–595
15. Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozden, E., Tuncman, G., Görgün, C., Glümer, L. H., and Hotamisligil, G. S. (2004) Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 306, 457–461
16. Zhou, L., Liu, M., Zhang, J., Chen, H., Dong, L. Q., and Liu, F. (2010) DsbA-L alleviates endoplasmic reticulum stress-induced adiponectin down-regulation. Diabetes 59, 2809–2816
17. Buechler, C., Wanninger, J., and Neumeier, M. (2010) Adiponectin receptor binding proteins: recent advances in elucidating adiponectin signaling pathways. FEBS Lett. 584, 4280–4286
18. Cassese, A., Esposito, I., Fiori, F., Barbagallo, A. P., Paturzo, F., Mirra, P., Ulianich, L., Giacco, F., Iadicicco, P., Lombardi, A., Oriente, F., Van Obberghen, E., Beguinot, F., Formisano, P., and Miele, C. (2008) In skeletal muscle advanced glycation end products (AGEs) inhibit insulin action and induce the formation of multimolecular complexes including the receptor for AGEs. J. Biol. Chem. 283, 36088–36099
19. Lee, M. H., Klein, R. L., El-Shewy, H. M., Luttrell, D. K., and Luttrell, L. M. (2008) The adiponectin receptors AdipoR1 and AdipoR2 activate ERK1/2 through a Src/Ras-dependent pathway and stimulate cell growth. Biochemistry 47, 11682–11692
20. Nakatani, Y., Kaneto, H., Kawamori, D., Yoshiuchi, K., Hatazaki, M., Matzuoka, T. A., Ozawa, K., Ogawa, S., Hori, M., Yasumaki, Y., and Matsushita, M. (2005) Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. J. Biol. Chem. 280, 847–851
21. Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, R. O., Görgün, C. Z., and Hotamisligil, G. S. (2006) Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 313, 1137–1140
22. Avignon, A., Yamada, K., Zhou, X., Spencer, B., Cardona, O., Saba-Sidique, S., Galloway, L., Standeart, M. L., and Faraese, R. V. (1996) Chronic activation of protein kinase C in soleus muscles and other tissues of insulin-resistant type II diabetic Goto-Kakizaki (GK), obese/aged, and obese/Zucker rats: a mechanism for inhibiting glycogen synthesis. Diabetes 45, 1396–1404
23. Kanoh, Y., Bandyopadhyay, G., Sajan, M. P., Standaert, M. L., and Farese, R. V. (2001) Rosiglitazone, insulin treatment, and fasting correct defective activation of protein kinase C-ζ/δ by insulin in vastus lateralis muscles and adipocytes of diabetic rats. Endocrinology 142, 1595–1605
24. Kellerer, M., Mushack, J., Seffer, E., Mischak, H., Ullrich, A., and Häring, H. U. (1998) Protein kinase C isoforms α, δ, and θ require insulin receptor substrate-1 to inhibit the tyrosine kinase activity of the insulin receptor in human kidney embryonic cells (HEK 293 cells). Diabetologia 41, 833–838
25. Rosenzweig, T., Braiman, L., Bak, A., Alt, A., Kuroki, T., and Sampson, S. R. (2002) Differential effects of tumor necrosis factor-α on protein kinase C isoforms α and δ mediate inhibition of insulin receptor signaling. Diabetes 51, 1921–1930