Phosphorylation of IRS proteins, insulin action, and insulin resistance

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> Boura-Halfon S, Zick Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. Am J Physiol Endocrinol Metab 296: E581-E591, 2009. First published August 28, 2008; doi:10.1152/ajpendo.90437.2008.—Insulin signaling at target tissues is essential for growth and development and for normal homeostasis of glucose, fat, and protein metabolism. Control over this process is therefore tightly regulated. It can be achieved by a negative feedback control mechanism whereby downstream components inhibit upstream elements along the insulin-signaling pathway (autoregulation) or by signals from apparently unrelated pathways that inhibit insulin signaling thus leading to insulin resistance. Phosphorylation of insulin receptor substrate (IRS) proteins on serine residues has emerged as a key step in these control processes under both physiological and pathological conditions. The list of IRS kinases implicated in the development of insulin resistance is growing rapidly, concomitant with the list of potential Ser/Thr phosphorylation sites in IRS proteins. Here, we review a range of conditions that activate IRS kinases to phosphorylate IRS proteins on "hot spot" domains. The flexibility vs. specificity features of this reaction is discussed and its characteristic as an "array" phosphorylation is suggested. Finally, its implications on insulin signaling, insulin resistance and type 2 diabetes, an emerging epidemic of the 21st century are outlined.

insulin receptor substrate

INSULIN RESISTANCE IS A STATE in which the sensitivity of target cells to respond to ordinary levels of insulin is reduced. It plays a central role in the development of type 2 diabetes, an emerging epidemic of the 21st century. A variety of agents and conditions that induce insulin resistance, such as TNF α and free fatty acids, activate a number of protein kinases that target elements along the insulin-signaling pathway. Some of these kinases phosphorylate the insulin receptor substrate (IRS) proteins. Ser/Thr phosphorylation of IRS proteins inhibits their function and interferes with insulin signaling in a number of ways (Fig. 1), thus leading to the development of an insulin resistance state.

In this review, we focus on the key molecular links between Ser/Thr phosphorylation of IRS proteins and the impairment in insulin signal transduction. We outline the relations between inflammation, stress responses, the activation of IRS kinases, and the induction of insulin resistance, and we propose a few directions for future studies in this field.

Insulin Signaling

Insulin is one of the anabolic hormones which promotes proper metabolism, energy balance and maintenance of normal body weight (56). Binding of insulin to its receptor activates the intrinsic tyrosine kinase activity of the receptor (IRK), which phosphorylates Tyr residues of target proteins such as the insulin receptor substrates (IRS-1 to -6), Shc proteins, Cbl, p60dok, APS, and Gab-1 (92, 112). Three major signaling pathways are propagated in response to activation of the IRK: phosphatidylinositol 3-kinase (PI3K), MAP kinase, and the Cbl/CAP pathway (92). The MAP kinase cascade leads to enhanced cell growth, while the Cbl/CAP cascade mediates glucose transport through activation of the GTP-binding protein TC10 and the recruitment of the CIP4/Gappex-5 complex to the plasma membrane (73). The PI3K cascade is activated by the IRS proteins to trigger the metabolic functions of insulin. Tyr-phosphorylated IRS proteins, the major insulin receptor substrates, function as signaling scaffolds that propagate insulin action through binding of Src homology 2 (SH2) domain-containing proteins. These include the p85 regulatory subunit of PI3K, Nck, Fyn, Grb-2, and SHP2, which mediate various aspects of insulin action (58, 64).

PI3K is one of the best-characterized downstream effector of IRS proteins (14). It associates with Tyr-phosphorylated IRS proteins following insulin stimulation and catalyzes the formation of phosphatidylinositol-3,4,5-trisphosphate, which stimulates phosphoinositide-dependent kinase (PDK-1) activity and initiates the activation of its downstream effectors protein kinase B (PKB, Akt), mammalian target of rapamycin (mTOR), and p70 S6 kinase (S6K1) as well as the atypical isoforms of PKC (PKC ζ/λ), leading to glucose transport and protein and glycogen synthesis (8, 108).

Control over insulin signaling can be achieved by autoregulation, whereby downstream components inhibit upstream elements (negative feedback control) (39, 80). Alternatively, signals from apparently unrelated pathways can inhibit insulin signaling. The insulin receptor (IR) and the IRS proteins are targets for such feedback control mechanisms (53, 72, 116, 117), with phosphorylation of IRS proteins on Ser residues being a key step in these feedback control processes (39, 80, 116, 117). Many of the insulin-stimulated Ser/Thr kinases that

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Fig. 1. Potential consequences of Ser/Thr phosphorylation of IRS proteins. IRS proteins contain a conserved pleckstrin homology (PH) domain at their NH2 terminus followed by a phosphotyrosine-binding (PTB) domain. The latter promotes interaction of IRS proteins with the juxtamembrane (JM) domain of the insulin receptor (IR), which phosphorylates the IRS proteins at their COOH-terminal region. Tyr-phosphorylated IRS proteins then serve as docking molecules for downstream effectors such as PI3K and phosphotyrosine phosphatase 2 (SHP-2). Prolonged insulin stimulation and other stimuli triggered by inducers of insulin resistance activate IRS kinases that phosphorylate the IRS proteins, such as IRS-1, on Ser/Thr residues. Such phosphorylation has several consequences. A: Ser phosphorylation of residues located at the PH domain interferes with binding of IRS-1 to the plasma membrane and to the receptor. B and C: phosphorylation of Ser/Thr residues within the PTB domain or at the COOH-terminal region of IRS-1 induces dissociation of IRS-1 from the receptor concomitant with IRS-1 degradation. D: phosphorylation of Ser residues at the COOH-terminal region interrupts with binding of IRS-1 to its downstream effectors.



are downstream effectors of IRS proteins serve as negative modulator of IRS proteins function. The activity of these kinases is blocked by inhibitors of the PI3K pathway, implicating Ser/Thr kinases, downstream of PI3K as potential IRS kinases (72). Interestingly, it is becoming apparent that inducers of insulin resistance such as tumor necrosis factor- α (TNF α), free fatty acids (FFAs), and cellular stress make use of similar mechanisms by activating a set of IRS kinases that phosphorylate the IRS proteins and inhibit their function (109, 115).

IRS Kinases and Insulin Resistance

Insulin resistance is defined as the failure of ordinary levels of insulin to trigger its downstream metabolic actions and is closely associated with obesity and the development of type 2 diabetes (55). It is becoming clear that obesity promotes a state of chronic low-grade inflammation and insulin resistance (51, 52, 98). This is attributed to the release from the adipose tissue of FFA, glycerol, hormones (e.g., leptin, adiponectin, endothelin-1), proinflammatory cytokines (e.g., TNF α , IL-1 β , IL-6), and additional products of macrophages that populate adipose tissue in obesity (27, 98, 109). It has been shown already a hundred years ago that high doses of salicylates lower glucose levels in diabetic patients implicating the involvement of inflammation in type 2 diabetes. Additionally, increased release of FFAs decreases insulin-mediated glucose transport in skeletal muscle and impairs suppression of glucose production by the liver, a characteristic of insulin resistance (3, 9, 47).

Many inducers of insulin resistance activate IRS kinases that negatively regulate insulin signaling and action. The list of IRS kinases implicated in the development of insulin resistance is growing rapidly, concomitant with the list of potential Ser/Thr phosphorylation sites in IRS proteins (22, 117). Recent studies have focused on IRS-1 as a major target for IRS kinases (22). However, it is now becoming evident that IRS-2 serves as a target as well (40, 97, 99). IRS kinases can be divided into two groups. One includes kinases that are mediators of insulin signaling. These kinases negatively regulate IRS proteins upon prolonged insulin stimulation [e.g., mTOR/S6K1 (105), MAPK (21), and PKCζ (65, 72, 90, 100)]. The other group consists of kinases that are activated along unrelated pathways to inhibit insulin action [e.g., glycogen synthase kinase (GSK)-3β (25, 69) IKKβ (33), c-Jun NH₂-terminal kinase (JNK) (2, 66), mouse Pelle-like kinase (mPLK) (59), and AMPK (103)]. Of note, several IRS kinases (e.g., S6K1, PKC) are activated both in response to insulin and as inducers of insulin resistance (80, 93, 104, 115).

Ser Phosphorylation of IRS Proteins and Its Potential Consequences

IRS proteins share a similar structure characterized by the presence of an NH2-terminal pleckstrin homology (PH) domain adjacent to a phosphotyrosine-binding (PTB) domain followed by a variable-length COOH-terminal tail that contains a number of Tyr and Ser phosphorylation sites. The PH domain is critical for IR-IRS interactions. Plasma membrane phospholipids, cytoskeletal elements, and protein ligands mediate these interactions (31, 37). In contrast, the PTB domain interacts directly with the juxtamembrane (JM) domain of the insulin and IGF-I receptors (88, 107), and hindrance of these interactions (by Ser/Thr phosphorylation) negatively affects insulin signaling (107). A third domain, the kinase regulatory loop binding (KRLB) is found only in IRS-2 (43, 95). This domain interacts with the phosphorylated regulatory loop of the IR, whereas the phosphorylation of two Tyr residues within the KRLB are crucial for this interaction (94).

The COOH-terminal end of each IRS protein contains a set of Tyr phosphorylation sites that act as on/off switches to recruit downstream effector molecules. IRS-1 and IRS-2 have the longest tails, which contain \sim 20 potential Tyr phosphorylation sites. Many of the Tyr residues gather into common Tyr-phosphorylated consensus motifs (YMXM or YXXM) that bind SH2 domains of their effector proteins. Ser/Thr phosphorylation adjacent to these Tyr phosphorylation sites impedes binding of the SH2 domains of these effectors, thus inhibiting insulin signaling.

Effects of autologous vs. "cross-talking" signaling cascades on IRS kinases that phosphorylate the PTB domain, leading to the dissociation of IRS proteins from the JM domain of IR. An obvious necessity for a successful protein-protein binding is a spatial matching. Therefore, it is becoming apparent that Ser/ Thr phosphorylation of IRS proteins in close proximity to their PTB (receptor-binding) region affects insulin signaling. We could show that mutation of seven Ser sites located within or in close proximity to the PTB domain of IRS-1 protects it from autologous desensitization as well as desensitization induced by IRS kinases triggered by inducers of insulin resistance (71). Autologous desensitization is exemplified by the atypical PKCζ, which is activated in response to insulin to mediate glucose uptake in adipocytes (7) and skeletal muscle (12, 70) downstream of IRS-1 and PI3K (70). In addition to its role as a mediator of insulin action, PKC ζ is involved in a selfattenuated mechanism induced by insulin to negatively regulate the function of IRS proteins upon prolonged insulin stimulation (72, 90). It involves Ser/Thr phosphorylation of IRS proteins, mediated by PKC ζ , which leads to the dissociation of IR:IRS (86) and IRS:PI3K (81) complexes. This inhibits the ability of IRS proteins to undergo further insulin-stimulated Tyr phosphorylation and, as a result, terminates insulin signaling (72). A direct interaction between IRS-1 and PKC was demonstrated in rat adipose tissue (90), implicating PKC as an IRS-1 kinase. The time line of action of PKCζ is still unclear. It is conceivable to assume that PKC ζ acts first on its target proteins along the glucose transport machinery to stimulate this process and promote insulin action before it acts on IRS-1 to dissociate it from the receptor and thus terminate insulin action.

Support for this conclusion is provided by studies (30) where muscle-specific knockout of PKC λ , a postulated mediator for insulin-stimulated glucose transport, was accompanied by systemic insulin resistance, impaired glucose tolerance, and islet β -cell hyperplasia while maintaining intact insulin signaling and actions in muscle, liver, and adipocytes of these mice. These findings demonstrate the importance of aPKC in insulinstimulated glucose transport in muscles of intact mice (28, 29). They further demonstrate that the stimulatory roles of aPKCs in insulin action override their inhibitory actions. They might further suggest that the inhibitory actions of PKC λ on insulin signaling have been overtaken by another kinase. Still, the molecular mechanism that coordinates these contradictory actions of atypical PKCs remains to be explored.

Ser³¹⁸ of IRS-1 (numbering of Ser sites is based on mouse IRS-1 and IRS-2 sequences unless otherwise indicated), is a potential target for PKC ζ (78), JNK, and kinases along the PI3K-mTOR pathway (82). It is located in close proximity to the PTB domain. Therefore, its phosphorylation presumably disrupts the interaction between IR and IRS-1. Phosphorylation of Ser³¹⁸ is not restricted to insulin stimulation. Elevated plasma levels of leptin, an adipokine produced by adipocytes (4), also stimulates the phosphorylation of Ser³¹⁸. This down-regulates insulin-stimulated Tyr phosphorylation of IRS-1 and glucose uptake (44).

Desensitization of insulin signaling is also triggered by several unrelated signaling cascades. Two major cascades are activated in response to inflammatory signals: one is mediated by the stress-activated JNK, and the other is mediated by IKK β (114). JNK1 promotes the phosphorylation of Ser³⁰⁷ of IRS-1 in response to TNF α (1, 2). Ser³⁰⁷ is adjacent to the PTB domain of IRS-1, and its phosphorylation interferes with the interaction of IR and IRS-1, thus preventing Tyr phosphorylation of IRS-1 (1). Indeed, JNK1-deficient mice show decreased adiposity and significantly improved insulin sensitivity (49). Palmitic acid induces JNK activation in pancreatic β -cells, resulting in the inhibition of pivotal gene transcription, including the insulin gene (99). This inhibition results in part due to phosphorylation of IRS-1 at Ser³⁰⁷ and of IRS-2 at Thr³⁴⁷, which most likely are inhibitory Ser/Thr sites (99).

Signaling via NF-kB is another key process during inflammation. IKK β is a Ser/Thr kinase that is part of the IKK complex that phosphorylates the inhibitor of NF-KB, IKB. This results in degradation of IkB, allowing the activation of NF-kB (57). Heterozygous deletion of IKK β (IKK $\beta^{+/-}$) protects against the development of insulin resistance during high-fat diet (HFD) and in obese Lep^{ob/ob} mice (61, 114). These findings support a pivotal role for IKK β in the induction of insulin resistance and diabetes. IKKB exerts its effects both globally (systemic) and locally (in selected tissues). Mice whose IKKB was selectively deleted from their myeloid cells preserve their whole body insulin sensitivity and are protected from insulin resistance induced by HFD (5). Similarly, hepatic expression of the I κ B α superrepressor reverses the type 2 diabetes phenotype induced by low-level activation of NF-KB (13). In contrast, selective loss of IKK β in hepatocytes retains insulin sensitivity in liver but not in muscle or fat tissues in response to HFD (114). The reason why expression of $I\kappa B\alpha$ superrepressor, but not deletion of IKKB in hepatocytes, reverses systemic insulin sensitivity of mice on HFD is not clear but could be attributed to different strategies employed to

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attenuate IKKB signaling. Nonetheless, IKKB seems to have a central role in hepatic insulin resistance (114) and in the development of systemic insulin resistance (13). At the molecular level, studies have yielded conflicting results. Interactions between IKKβ and IRS-1 were shown, whereas Ser³⁰⁷ was implicated as a potential phosphorylation site for IKK β (20, 33). Support for this conclusion was provided by showing that the motor protein Myo1c and its receptor protein NEMO act cooperatively to form the IKKB:IRS-1 complex that functions in TNF α -induced insulin resistance (83). In contrast, a reduction in IKKβ levels using specific small interfering (si)RNA failed to prevent TNFa-mediated IRS-1 phosphorylation on Ser³¹² (human equivalent of mouse Ser³⁰⁷) in primary human skeletal muscle (6). This apparent discrepancy could be attributed to the fact that Ser³⁰⁷ is subjected to phosphorylation by a number of IRS kinases in addition to IKKB (Fig. 2) Furthermore, IKK β can phosphorylate sites different from Ser³⁰⁷. We have shown that mutations of seven Ser sites in IRS-1 (Ser^{265/302/325/336/358/407/408}) confer protection from the action of IKK β when the mutated IRS-1 (IRS-1^{7A}) is overexpressed in Fao cells or primary hepatocytes (46). Interestingly, Ser³⁰⁷ was not one of the seven mutated sites in IRS-1^{7A}, implicating other Ser residues as potential IKKβ-mediated phosphorylation sites.

Inducers of insulin resistance also activate PKC θ , a noveltype PKC. PKC θ is activated upon increased content of intramuscular long-chain fatty acyl-CoA. An increase in PKC0 activity occurs concomitantly with a decrease in insulin-stimulated Tyr phosphorylation of IRS-1 and a reduction in glucose transport (38, 113). PKC θ -deficient mice are protected against fat-induced defects in insulin signaling (e.g., reduced insulinstimulated Tyr phosphorylation of IRS-1 and glucose uptake) in skeletal muscle, further supporting the role of PKC θ in mediating fatty acid-induced insulin resistance (60). Notably, in the latter study, no direct evidence on Ser phosphorylation of IRS-1 or activation of other Ser/Thr kinases such as IKKβ or JNK was provided. The notion that PKC θ plays a role in fatty acid-mediated insulin resistance was challenged by the findings that transgenic mice with muscle-specific expression of a dominant negative PKC0 have shown age- and obesity-associated glucose intolerance, implicating PKC θ as a protective rather than a negative regulator of insulin function (96). These discrepant findings may be due to the different technical approaches that were used. For example, a dominant negative PKC θ could bind (and titrate out) proteins necessary for the proper function of other PKC isoforms. At the molecular level, PKC θ , a known activator of IKK β and JNK (60, 101), may attenuate insulin signaling directly or via the activation of these IRS kinases. Ser¹¹⁰¹ (68) and Ser³⁰⁷ (113) were suggested as potential target sites of PKC0 in IRS-1.

Autologous vs. heterologous feedback mechanisms can sometimes act in antagonistic manner. This is exemplified by GSK-3, an important mediator of insulin signaling, which phosphorylates IRS proteins on Ser residues to attenuate insulin actions. Ser³³² in IRS-1 is a potential GSK-3 phosphorylation site, with Ser³³⁶ being the priming site (69). GSK-3 activity is inhibited by insulin upon its phosphorylation by insulin-stimulated PKB (19). Hence, GSK-3 activity is not part of an autologous insulin-induced negative feedback control mechanism. In contrast, inducers of insulin resistance stimulate the activity of GSK-3 (18, 45), which is elevated in diabetic tissues (26). Accordingly, GSK-3a KO mice display enhanced glucose and insulin sensitivity accompanied by reduced fat mass. Hepatic insulin signaling in these mice is increased, and so is the IRS-1 expression (76). Moreover, a reduction in GSK-3 α in human muscle cells results in an increase in insulin-stimulated glucose uptake, glycogen synthase activity, and IRS-1 expression (16), whereas treatment with GSK-3 inhibitors enhances insulin actions in vitro and in vivo (17, 85). Of note, insulin-mediated phosphorylation of PKB and glycogen synthase were similar in skeletal muscle from both wildtype and GSK-3α KO mice, which may indicate different roles for GSK-3 α in mice vs. human (16, 76). Similarly, McManus et al. (77), have generated homozygous knock-in mice of constitutively active (CA) GSK- 3α , GSK- 3β , or GSK- $3\alpha\beta$. Although GSK-3β had a major role in regulating glycogen synthase activity in muscle of CA GSK-3^β knock-in mice, these animals were not diabetic, and their insulin-stimulated PKB activation and glucose uptake were not changed. These findings cast a certain doubt as to the critical role of GSK-3 β in the attenuation of insulin signaling, at least in mouse models, although constitutive activation of GSK-3β under normal conditions (e.g., regular diet, low BMI) might be necessary, but it is insufficient to promote systemic insulin resistance. Hence, with respect to GSK-3, insulin acts to prevent its action as an IRS kinase, whereas heterologous signaling cascades activate it.

Interference with IRS: IR complex formation mediated by the PH domain. IRS kinases, triggered by inducers of insulin resistance, can phosphorylate sites located within the PH domain of IRS proteins. An example is the mouse Pellet-like kinase (mPLK, homolog of human IL-1 receptor-associated kinase) (59). Overexpression of mPLK-WT impairs, apparently via Ser²⁴ phosphorylation, insulin-stimulated Tyr phosphorylation of IRS-1 and its association with p85 α . Ser²⁴, located within the PH domain of IRS-1, seems to be critical for IR:IRS-1 complex formation (31). Interestingly, insulin can also trigger conventional and novel PKCs that phosphorylate this site. Conventional and novel PKCs were shown to be activated by insulin under certain conditions (91), and their role as transducers and modulators of insulin signaling is discussed in a recent review (93). Ser²⁴ is a potential phosphorylation site for PKC α (84). Similarly, insulin-stimulated phosphorylation of Ser²⁴, apparently by PKCδ, diminishes the ability of IRS-1 to bind phosphatidylinositol-4,5-bisphosphate (PIP₂) further supporting the hypothesis that Ser^{24} is a negative regulatory phosphorylation site in IRS-1 (36). In agreement with this prediction, knockout of PKCa enhances insulin signaling (67). In a recent study using mass spectrometry analysis, Ser⁶⁷ (rat numbering), located at the PH domain of IRS-1, was shown to be phosphorylated upon prolonged insulin stimulation. This site as well may be considered an inhibitory site (34). Consequently, Ser phosphorylation within the PH domain of IRS proteins could account for the development of insulin resistance state.

Inhibiting the ability of downstream effectors to dock and bind to specific tyr residues at the COOH-terminal tail of IRS proteins. Many of the Tyr-phosphorylated consensus motifs (YMXM or YXXM) of IRS-1 are located at its COOHterminal tail. Consequently Ser phosphorylation within this area could interrupt with the binding of downstream effector proteins of IRS-1 such as $p85\alpha$ and the phosphotyrosine

PHOSPHORYLATION OF IRS PROTEINS

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IRS-1 phosphorylation on Ser residues

Fig. 2. A: Ser phosphorylation of IRS-1: is it an array phenomenon? Representative list of Ser residues of IRS-1 that were shown to undergo phosphorylation as a result of different stimuli. B: Ser/Thr phosphorylation of IRS-2. IRS-1/2 kinases (*middle*) are activated by several stimuli (*left*) to phosphorylate IRS-1/2 on a number of Ser/Thr residues (*right*). *Mouse numbering. **Sites marked with star were mutated as a group. ***5-Aminoimidazole-4-carboxamide riboside. Black, blue, and red filled circles represent, respectively, a suggested negative, positive, or negative/positive regulatory phosphorylation site.

B

A

IRS-2 phosphorylation on Ser/Thr residues



phosphatase SHP-2. Indeed, Ser⁵⁷⁰ of IRS-1, located in the vicinity of the PI3K interaction motif, was shown to be a potential PKC ζ phosphorylation site that, upon phosphorylation, disrupts the IRS-1-p85 α complex (31). IRS-3 and IRS-4 but not IRS-2 are also substrates for PKC ζ (65). Potential MAP kinase phosphorylation sites, Ser⁶¹², Ser⁶³², Ser⁶⁶², and Ser⁷³¹ in IRS-1, located next to Tyr phosphorylation YMXM motifs,

were shown to be negative regulators for PI3K activity associated with IRS-1 (81). A twofold increase in the basal phosphorylation of IRS-1 on Ser⁶³⁶ (human numbering) was observed in muscle biopsies from patients with type 2 diabetes, concomitantly with higher basal activity of extracellular signalregulated kinase (ERK)1/2. Defects in insulin signaling were evident, including reduced PI3K activity, decreased association of PI3K with IRS-1, and reduced Tyr phosphorylation of IRS-1 during insulin stimulation. Inhibition of ERK1/2 by a specific inhibitor strongly inhibited Ser⁶³⁶ phosphorylation, thus implicating ERK1/2 in the phosphorylation of Ser⁶³⁶ and in the attenuation of insulin signaling (11).

mTOR and S6K1 kinases are downstream effectors of PI3K and potential candidates for negative regulation of IRS proteins. Indeed, mTOR complex 1 (mTORC1), an integrator of nutrient and insulin signaling, and its downstream target S6K1 are critical components in mediating the nutrient effects on insulin resistance (15, 63). Um et al. (104) clearly demonstrated that S6K1 negatively modulates insulin's effects by phosphorylating IRS proteins. The pivotal role played by S6K1 is indicated by the fact that when $S6K1^{-/-}$ mice are placed on HFD, their levels of glucose and FFA rise, and they fail to fully autophosphorylate and activate their IRs; still, they maintain their capacity to activate downstream effectors such as PKB, as opposed to their WT counterpart. S6K1-deficient mice remain sensitive to insulin, owing to apparent loss of a negative feedback loop from S6K1 to IRS-1, which blunts Ser³⁰⁷ and Ser^{636/639} phosphorylation; sites involved in insulin resistance (105). In a recent publication, Ser^{1101} was identified as another S6K1 site in IRS-1, the phosphorylation of which is increased upon nutrient overload and obese setting. Phosphorylation of Ser¹¹⁰¹ was increased in liver of obese (db/db) or WT but not of S6K1^{-/-} mice maintained on HFD, implicating S6K as the kinase involved (102). The potential Ser residues implicated in the negative feedback regulation of S6K1 are located at the PTB domain [Ser³⁰⁷, Ser³⁰² (41)] and in close proximity to Tyr-phosphorylated consensus motifs at the COOH-terminus of IRS-1 (Ser^{636/639}). Such phosphorylation leads to dissociation of IR:IRS complexes concomitant with an inhibition of downstream effectors to dock and bind to IRS-1. The findings that persistent activation of mTOR/S6K might be responsible for impaired insulin actions lead to the obvious question whether inhibition of these kinases improves insulin sensitivity in insulin resistance states. Consistent with these findings, in a short-term in vivo study, in which insulin resistance was introduced by infusion of a high concentration of amino acids in humans, rapamycin, an mTOR inhibitor, improved insulin actions (62). On the other hand, long-term treatment with rapamycin increased rather than decreased the insulin resistance of *Psammomys obesus* that was induced by high-energy diet (32). This could be attributed to the increased activity of stress-response kinases in muscle and islets. Hence, either sustained activation of the mTOR/S6K1 pathways or their inhibition could result in insulin resistance albeit through different mechanisms.

An additional Ser site at the COOh terminus of IRS-1 is Ser⁷⁸⁹. Ser⁷⁸⁹ was shown to be a target for the salt-inducible kinase-2 (SIK2, a novel member of the AMPK family) in adipose cells (50). The activity and content of SIK2 are elevated in white adipose tissue of *db/db* diabetic mice, suggesting that overexpression of SIK2 induces phosphorylation of Ser⁷⁸⁹ of IRS-1 and as a result negatively regulates insulin signal transduction (50). Tzatsos et al. (103) could show that AMPK is activated upon energy depletion (glucose deprivation, hypoxia) to phosphorylate IRS-1 on Ser⁷⁹⁴ (human equivalent of mouse Ser⁷⁸⁹), which further inhibits PI3K/Akt signaling. Similarly, it was suggested that SIK2 may function like AMPK to turn off lipogenesis in low-energy states (23). Qiao

et al. (89), however, claim that an unknown Ser/Thr kinase rather than AMPK phosphorylates IRS-1 on Ser⁷⁸⁹ in an insulin-resistant animal model. In contrast, phosphorylation of this site was shown to promote insulin signaling (54) in the muscle C_2C_{12} cell line. The latter study invovled the use of AICAR (5-aminoimidazole-4-carboxamide riboside), a selective inducer of AMPK, and showed a correlation between Ser⁷⁸⁹ phosphorylation of IRS-1 and activation of PI3K. Still, we cannot rule out the possibility that AMPK activates IRS-1 by a different mechanism while the phosphoryaltion of Ser⁷⁸⁹ reflects a delayed inhbitory response.

Induction of degradation of IRS proteins. Sustained insulin treatment that resembles hyperinsulinemia in insulin resistance states induces IRS-1 degradation. Downstream targets of the PI3K and mTOR pathways were implicated in mediating IRS-1 phosphorylation under these conditions (24, 42, 48). Activation of G protein-coupled receptor kinase-2 (GRK-2) upon chronic endothelin-1 treatment induces phosphorylation and degradation of IRS-1, most likely at Ser⁶¹² (53, 106). Insulin-stimulated degradation of IRS-1 via the PI3K pathway depends in part on phosphorylation of Ser³⁰⁷. Indeed, a mutated form of IRS-1, in which Ser³¹² (human equivalent of mouse Ser³⁰⁷) was replaced with Ala is protected from insulin-stimulated IRS-1 degradation (37). Of note, phosphorylation of Ser³⁰⁷ might be necessary, but it is insufficient to promote IRS-1 degradation. In accord with this conclusion we could show that insulin-induced degradation of a mutated form of IRS-1 was significantly reduced even when Ser³⁰⁷ phosphorylation remained unchanged (10). We could further show that elimination of an entire Ser/Thr-rich domain of IRS-1, proximal to its PTB domain protects IRS-1 from chronic-insulin induced degradation (10). Collectively, these findings support the notion that Ser/Thr phosphorylation is involved in insulin-stimulated IRS-1 degradation.

Positive regulation of IRS-1 by insulin-mediated ser phos*phorylation*. Tyr phosphorylation of IRS-1 positively regulates IRS-1 activity. Ser phosphorylation mainly negatively regulates IRS-1 function, as discussed above, although it might have some positive roles. The first indication linking Ser phosphorylation of IRS-1 and improved IRS-1 function (e.g., increased Tyr phosphorylation) was observed (87) when an IRS-1 mutant lacking four potential PKB phosphorylation sites (Ser²⁶⁵, Ser³⁰², Ser³²⁵, Ser³⁵⁸) markedly enhanced the rate of Tyr dephosphorylation, implicating one or more of these sites as positive regulator of IRS-1 function (87). In agreement with these findings, Ser³⁰² phosphorylation was implicated as a positive mediator of nutrient availability that promotes mitogenesis and cell growth (35). Using mass spectrometry techniques, Luo et al. (75) could demonstrate that phosphorylation of Ser¹²²³ or Ser⁶²⁹ (human numbering) resulted in an increased IRS-1 function in respond to insulin. Two distinct mechanisms were suggested. Phosphorylation of Ser¹²²³ reduces association of IRS-1 with SHP-2, a Tyr phosphatase, thereby increasing IRS-1 Tyr phosphorylation. Phosphorylation of Ser⁶²⁹ was proposed to attenuate the phosphorylation of a second Ser site, Ser⁶³⁶, which was implicated as a negative regulator of insulin signaling, and thereby phosphorylation of Ser⁶²⁹ enhanced insulin signaling (74).

These findings suggest that, upon acute insulin stimulation, IRS-1 is phosphorylated on Tyr residues to propagate insulin signaling, a reaction accompanied by its phosphorylation on

Ser residues, which serve as "guardians" of the phosphorylated Tyr residues, namely, by inhibiting Tyr phosphatases and/or phosphorylation at "inhibitory" Ser sites. Phosphorylation of IRS-1 on these inhibitory Ser sites then commences with a delayed onset.

Phosphorylation of IRS Proteins in Human Subjects

Most information related to the regulation of IRS protein function is based on studies in cell cultures and in mouse models. Still, accumulating evidence of in vivo studies in humans supports the concept that increased Ser/Thr phosphorylation of IRS proteins might turn subjects prone to the development of insulin resistance (80). The IRS kinases involved seem to be those already implicated as negative regulators of insulin signaling. In a recent study using skeletal muscle biopsies from 11 humans, the mTOR-S6K pathway was shown to negatively modulate glucose metabolism under nutrient abundance (62). In agreement with previous studies, phosphorylation of Ser³¹² and Ser⁶³⁶ of IRS-1 was implicated as part of this negative regulation (62, 102). Increased phosphorylation of Ser⁶³⁶ of IRS-1 was observed in myotubes of patients with type 2 diabetes. Inhibition of ERK1/2 with PD-98059 reduced this phosphorylation, thereby implicating ERK1/2 in the phosphorylation of Ser^{636} in human muscle (11).

To unveil the importance of phosphorylated Ser/Thr residues of human IRS-1, Yi et al. (111) adopted a mass spectrometry approach. Although this system may suffer some drawbacks, a number of the potential Ser/Thr residues involved in the regulation of IRS proteins function could be identified. More than 20 Ser residues of IRS-1 were found to undergo insulinstimulated phosphorylation in human muscle biopsies, three of which were newly identified sites: Thr⁴⁹⁵, Ser⁵²⁷, and Ser¹⁰⁰⁵ (human numbering). This report validates previous in vitro and in vivo studies in animal models and suggests that the same strategy could be employed to identify phosphorylated Ser/Thr sites under conditions of insulin resistance, obesity, or type 2 diabetes.

Ser Phosphorylation of IRS Proteins: Is It an Array Phenomenon or an Event Confined to Few Selected Ser Sites?

Data collected so far suggest that an array of Ser/Thr phosphorylation sites of IRS-1 attenuates insulin signaling rather than phosphorylation of single selected sites (Fig. 2). Ser/Thr phosphorylation disrupts at least three interactions of IRS-1: with the plasma membrane, the receptor, or its downstream effectors. Therefore, many of the potential Ser residues distributed along the IRS proteins could be involved. IRS-1 contains more than 70 Ser residues at potential consensus phosphorylation sites, and each signal could induce the phosphorylation of a different set of residues. Hence, numerous optional phosphorylation combinations are possible. To obtain an accurate analysis, all potential sites phosphorylated in response to various stimuli should be examined as opposed to analysis of a small number of residues limited by availability of phosphospecific antibodies. It has already been shown that quite a number of Ser residues undergo phosphorylation upon insulin treatment in human muscle biopsies (111) and in CHO^{IR/IRS} cells (34), indicating a robust phosphorylation of an array of Ser sites of IRS-1 in an in vivo setting. In both studies, Ser/Thr phosphorylation of IRS-1 was analyzed upon prolonged insulin stimulation (30-120 min) using mass spectrometry, and around 20 sites were suggested as potential phosphorylated sites in each study. Of note, these lists were not identical, thus suggesting that an even longer list of Ser sites are phosphorylated upon insulin stimulation or upon treatment with inducers of insulin resistance.

Specificity vs. Flexibility

The identity of the Ser residues, the phosphorylation of which results in specific alteration in IRS proteins structure and function is still poorly understood. Ser³⁰⁷ is considered to be involved in IRS-1 degradation (37), while we have shown that deletion of a Ser-rich domain of IRS-1 protects it from insulinstimulated degradation despite the fact that this IRS-1 mutant is still highly phosphorylated on Ser³⁰⁷ (10). We could also show that mutation of seven Ser sites of IRS-1, different from Ser³⁰⁷, confers upon the mutant protein protection from the inhibitory action of sustained insulin treatment or the inhibitory effects of proinflammatory cytokines (46, 71). These findings support the notion that phosphorylation of IRS proteins at selected domains (e.g., their PTB domain), rather than phosphorylation of selected sites (e.g., Ser³⁰⁷), is required to inhibit IRS protein function. Accordingly, mutations of seven Ser residues within a given domain confer stronger protection from IRS kinases than mutations of three or a single residue (46, 71). This conclusion is supported by a recent study that made use of muscle-specific knock-in mice that express a mutant IRS-1 where three Ser residues were replaced by alanine (Ser³⁰², Ser³⁰⁷, Ser⁶¹²) (79). The transgenic mice were partially protected from fat-induced insulin resistance. These studies therefore indicate that mutation of a number of Ser/Thr phosphorylation sites, rather than single site-specific mutation, sensitizes IRS-1 and protects it from negative feedback regulation of insulin signaling. The issue of dominance should also be considered, namely, what happens when an array of "positive" and "negative" Ser sites is mutated. For example, we could show that when such "mixed" mutation is performed the net effect is potentiation of insulin signaling (46, 71), suggesting the dominance of "inhibitory" Ser sites over the "stimulatory" sites in this particular case. All together, these findings indicate the existence of a cross talk between Ser phosphorylation sites and suggest that the overall phosphorylation pattern dictates **IRS-1** functions.

Ser/Thr Phosphorylation of IRS-2 Protein

Most of the data accumulated so far focus on Ser phosphorylation of IRS-1 rather than IRS-2. Although IRS-2 was cloned more than a decade ago, there were not too many studies of its regulation by Ser/Thr phosphorylation (*schema 2B*). Recently, it was shown that palmitic acid induces JNK activation in pancreatic β -cells, resulting in the inhibition of pivotal gene transcription, including insulin. This inhibition resulted partly due to phosphorylation of IRS-2 at Thr³⁴⁷, which was implicated as a potential inhibitory Thr site (99). Sequential in vitro phosphorylation of IRS-2 on Ser⁴⁸⁴ and Ser⁴⁸⁸ by GSK-3 and JNK, respectively, was suggested to promote hepatic insulin resistance (97). We recently mutated into Ala five potential inhibitory Ser sites located proximal to the PTB domain of IRS-2 (Ser³⁰³, Ser³⁴³, Ser³⁶², Ser³⁸¹, Ser⁴⁸⁰). We were able to

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show that cytokine-treated pancreatic islets overexpressing the mutated IRS-2 were protected from apoptosis and secreted significantly more insulin in response to glucose compared with islets overexpressing IRS-2^{WT}. Consequently, these five Ser residues can be considered negative regulators of IRS-2 function (40). IRS-2 function is also regulated by mechanisms distinct from Ser/Thr phosphorylation. For example, the kinase regulatory-loop binding (KRLB) region (aa 591–733), which is unique to IRS-2, serves as a negative regulatory element to control the extent of Tyr phosphorylation of IRS-2. Hence, inhibition exerted by KRLB domain attenuates insulin signaling and action independent of Ser/Thr phosphorylation (94, 110).

Future Perspectives

Impaired regulation of insulin signaling is a critical factor in the development of insulin resistance, and a better understanding of this process may lead to the development of novel therapies. IRS proteins are major targets for Ser/Thr phosphorylation-based negative regulation that uncouples them from their upstream receptors and their downstream effectors, leading to the termination of insulin signaling. A positive role for Ser phosphorylation was implicated as well; however, data on stimulatory Ser sites is less comprehensive and the underlying mechanisms are yet elusive.

It is becoming clear, however, that Ser phosphorylation of IRS proteins involves a number of IRS kinases. Hence, Ser/Thr phosphorylation of IRS proteins represents combinatorial consequences of several kinases, activated by different pathways, acting in concert to phosphorylate multiple sites to generate a rather complicated network. This model is supported by studies that show that one stimulus could increase the phosphorylation of many Ser residues in IRS-1. Second, elimination of a number of Ser residues of IRS-1 confers upon IRS-1 better protection from inducers of insulin resistance than elimination of single sites. Major questions that remain to be addressed are: which kinases directly phosphorylate IRS proteins, thereby affecting insulin signaling; which Ser residues are the most critical in regulating IRS function; and are there Ser/Thr-rich domains whose phosphorylation at a number of sites inhibits **IRS** proteins function?

The spatial and temporal regulatory elements that control this complex phosphorylation network need to be revealed. It is conceivable to assume that Ser kinases activated along the insulin pathway (e.g., S6K1, PKCζ) will be allowed first to execute their action as promoters of insulin signaling before they induce the phosphorylation of IRS proteins, as part of a negative feedback mechanisms that will terminate their own activation. Then, how these kinases are targeted toward their different substrates (e.g., S6 the "positive" signal vs. IRS-1 the "negative" signal) is currently unknown. The issue of stimulatory vs. inhibitory Ser sites of IRS-1 also needs further clarification. Does phosphorylation of stimulatory sites precede that of inhibitory sites; and if so, what regulates this process? The issue of "priming" deserves attention. For example, does phosphorylation of IRS proteins at stimulatory Ser sites "tag" the protein to further phosphorylation at inhibitory Ser sites?

The array of Ser/Thr phosphatases that dephosphorylate the different Ser residues and reset the system to its basal state have not been elucidated. It is only conceivable to assume that

the activity of these phosphatases is regulated to no lesser extent than the activity of the IRS kinases.

Several strategies could be applied to address these questions. The fast-developing field of mass spectrometry now enables the identification of arrays of Ser sites of IRS proteins that are subjected to phosphorylation under in vivo conditions. Samples of human tissues, processed to isolate their IRS proteins and subject them to such analysis, could be most insightful in defining the extent of phosphorylation of each site under physiological or pathological conditions.

Introduction of siRNA technology could help decipher the role of individual kinases, or a kinase combination thereof, in regulating IRS protein function. Combined with tissue-specific knockout of given kinases, this strategy could enlighten our understanding about the IRS kinases activated under different biological conditions. Then, knock-in into IRS-1/2-null mice of IRS proteins mutated at selected Ser phosphorylation sites should provide insight into the role of selected Ser sites in the regulation of IRS proteins function.

Addressing these and related questions awaits further studies that will lead us to a better understanding of this complex process. Such research has much clinical relevance and physiological importance, as it might direct us toward new potential therapeutic strategies to treat insulin resistance and diabetes.

REFERENCES

- Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* 275: 9047–9054, 2000.
- Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* 277: 1531–1537, 2002.
- Anderwald C, Roden M. Adipotoxicity and the insulin resistance syndrome. *Pediatr Endocrinol Rev* 1: 310–319, 2004.
- Argiles JM, Lopez-Soriano J, Almendro V, Busquets S, Lopez-Soriano FJ. Cross-talk between skeletal muscle and adipose tissue: a link with obesity? *Med Res Rev* 25: 49–65, 2005.
- Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11: 191– 198, 2005.
- Austin RL, Rune A, Bouzakri K, Zierath JR, Krook A. siRNAmediated reduction of nuclear factor-kappaB prevents TNF-alpha-induced insulin resistance in human skeletal muscle. *Diabetes* 57: 2066– 2073, 2008.
- Bandyopadhyay G, Sajan MP, Kanoh Y, Standaert ML, Quon MJ, Lea-Currie R, Sen A, Farese RV. PKC-zeta mediates insulin effects on glucose transport in cultured preadipocyte-derived human adipocytes. *J Clin Endocrinol Metab* 87: 716–723, 2002.
- Bandyopadhyay G, Standaert ML, Galloway L, Moscat J, Farese RV. Evidence for involvement of protein kinase C (PKC)-zeta and noninvolvement of diacylglycerol-sensitive PKCs in insulin-stimulated glucose transport in L6 myotubes. *Endocrinology* 138: 4721–4731, 1997.
- Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest* 32, *Suppl* 3: 14–23, 2002.
- Boura-Halfon S, Beck A, Petrovich K, Gurevitch D, Sasson K, Ronen D, Zick Y. A novel domain mediates post-ubiquitination-independent, insulin-induced proteasomal degradation of IRS-1. 3rd Russell Berrie D-Cure Symposium, Jerusalem, Israel, Diabetes and Obesity (Abstract Book 2007), p. 59.
- 11. Bouzakri K, Roques M, Gual P, Espinosa S, Guebre-Egziabher F, Riou JP, Laville M, Le Marchand-Brustel Y, Tanti JF, Vidal H. Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of

skeletal muscle cells from patients with type 2 diabetes. *Diabetes* 52: 1319–1325, 2003.

- Braiman L, Alt A, Kuroki T, Ohba M, Bak A, Tennenbaum T, Sampson SR. Activation of protein kinase C zeta induces serine phosphorylation of VAMP2 in the GLUT4 compartment and increases glucose transport in skeletal muscle. *Mol Cell Biol* 21: 7852–7861, 2001.
- Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 11: 183–190, 2005.
- Cantley LC. The phosphoinositide 3-kinase pathway. Science 296: 1655–1657, 2002.
- Carlson CJ, White MF, Rondinone CM. Mammalian target of rapamycin regulates IRS-1 serine 307 phosphorylation. *Biochem Biophys Res Commun* 316: 533–539, 2004.
- Ciaraldi TP, Nikoulina SE, Bandukwala RA, Carter L, Henry RR. Role of glycogen synthase kinase-3 alpha in insulin action in cultured human skeletal muscle cells. *Endocrinology* 148: 4393–4399, 2007.
- Cline GW, Johnson K, Regittnig W, Perret P, Tozzo E, Xiao L, Damico C, Shulman GI. Effects of a novel glycogen synthase kinase-3 inhibitor on insulin-stimulated glucose metabolism in Zucker diabetic fatty (falfa) rats. *Diabetes* 51: 2903–2910, 2002.
- 18. Cohen P, Frame S. The renaissance of GSK3. *Nat Rev Mol Cell Biol* 2: 769–776, 2001.
- Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378: 785–789, 1995.
- de Alvaro C, Teruel T, Hernandez R, Lorenzo M. Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitor kappaB kinase in a p38 MAPK-dependent manner. *J Biol Chem* 279: 17070–17078, 2004.
- De Fea K, Roth RA. Modulation of insulin receptor substrate-1 tyrosine phosphorylation and function by mitogen-activated protein kinase. *J Biol Chem* 272: 31400–31406, 1997.
- 22. Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85alpha: the two sides of a coin. *Diabetes* 55: 2392–2397, 2006.
- Du J, Chen Q, Takemori H, Xu H. SIK2 can be activated by deprivation of nutrition and it inhibits expression of lipogenic genes in adipocytes. *Obesity (Silver Spring)* 16: 531–538, 2008.
- Egawa K, Nakashima N, Sharma PM, Maegawa H, Nagai Y, Kashiwagi A, Kikkawa R, Olefsky JM. Persistent activation of phosphatidylinositol 3-kinase causes insulin resistance due to accelerated insulininduced insulin receptor substrate-1 degradation in 3T3-L1 adipocytes. *Endocrinology* 141: 1930–1935, 2000.
- Eldar-Finkelman H, Krebs EG. Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. *Proc Natl Acad Sci USA* 94: 9660–9664, 1997.
- Eldar-Finkelman H, Schreyer SA, Shinohara MM, LeBoeuf RC, Krebs EG. Increased glycogen synthase kinase-3 activity in diabetesand obesity-prone C57BL/6J mice. *Diabetes* 48: 1662–1666, 1999.
- Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology* 145: 2273–2282, 2004.
- Farese RV. Function and dysfunction of aPKC isoforms for glucose transport in insulin-sensitive and insulin-resistant states. *Am J Physiol Endocrinol Metab* 283: E1–E11, 2002.
- Farese RV, Sajan MP, Standaert ML. Atypical protein kinase C in insulin action and insulin resistance. *Biochem Soc Trans* 33: 350–353, 2005.
- Farese RV, Sajan MP, Yang H, Li P, Mastorides S, Gower WR Jr, Nimal S, Choi CS, Kim S, Shulman GI, Kahn CR, Braun U, Leitges M. Muscle-specific knockout of PKC-lambda impairs glucose transport and induces metabolic and diabetic syndromes. *J Clin Invest* 117: 2289–2301, 2007.
- Farhang-Fallah J, Randhawa VK, Nimnual A, Klip A, Bar-Sagi D, Rozakis-Adcock M. The pleckstrin homology (PH) domain-interacting protein couples the insulin receptor substrate 1 PH domain to insulin signaling pathways leading to mitogenesis and GLUT4 translocation. *Mol Cell Biol* 22: 7325–7336, 2002.
- 32. Fraenkel M, Ketzinel-Gilad M, Ariav Y, Pappo O, Karaca M, Castel J, Berthault MF, Magnan C, Cerasi E, Kaiser N, Leibowitz G. mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia

and exacerbates the metabolic state in type 2 diabetes. *Diabetes* 57: 945–957, 2008.

- 33. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, Ye J. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* 277: 48115–48121, 2002.
- Giraud J, Haas M, Feener EP, Copps KD, Dong X, Dunn SL, White MF. Phosphorylation of Irs1 at SER-522 inhibits insulin signaling. *Mol Endocrinol* 21: 2294–2302, 2007.
- Giraud J, Leshan R, Lee YH, White MF. Nutrient-dependent and insulin-stimulated phosphorylation of insulin receptor substrate-1 on serine 302 correlates with increased insulin signaling. *J Biol Chem* 279: 3447–3454, 2004.
- Greene MW, Ruhoff MS, Roth RA, Kim JA, Quon MJ, Krause JA. PKCdelta-mediated IRS-1 Ser24 phosphorylation negatively regulates IRS-1 function. *Biochem Biophys Res Commun* 349: 976–986, 2006.
- Greene MW, Sakaue H, Wang L, Alessi DR, Roth RA. Modulation of insulin-stimulated degradation of human insulin receptor substrate-1 by Serine 312 phosphorylation. J Biol Chem 278: 8199–8211, 2003.
- Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI. 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, 1999.
- 39. Gual P, Le Marchand-Brustel Y, Tanti J. Positive and negative regulation of glucose uptake by hyperosmotic stress. *Diabetes Metab* 29: 566–575, 2003.
- 40. Gurevitch D, Boura-Halfon S, Issac R, Alberstein M, Ronen D, Lewis E, Zick Y. Phosphorylation of selected serines at the PTB domain of IRS-2 regulates beta cell growth, survival, and insulin secretion. *In: Keystone Symposia on Islet and Beta-Cell Development and Transplantation* (2008 Abstract Book), p. 144.
- 41. Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, Barnett J, Leslie NR, Cheng S, Shepherd PR, Gout I, Downes CP, Lamb RF. The TSC1–2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. *J Cell Biol* 166: 213–223, 2004.
- Haruta T, Uno T, Kawahara J, Takano A, Egawa K, Sharma PM, Olefsky JM, Kobayashi M. A rapamycin-sensitive pathway downregulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol* 14: 783–794, 2000.
- 43. He W, Craparo A, Zhu Y, O'Neill TJ, Wang LM, Pierce JH, Gustafson TA. Interaction of insulin receptor substrate-2 (IRS-2) with the insulin and insulin-like growth factor I receptors. Evidence for two distinct phosphotyrosine-dependent interaction domains within IRS-2. *J Biol Chem* 271: 11641–11645, 1996.
- 44. Hennige AM, Stefan N, Kapp K, Lehmann R, Weigert C, Beck A, Moeschel K, Mushack J, Schleicher E, Haring HU. Leptin downregulates insulin action through phosphorylation of serine-318 in insulin receptor substrate 1. FASEB J 20: 1206–1208, 2006.
- 45. Henriksen EJ, Dokken BB. Role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. *Curr Drug Targets* 7: 1435–1441, 2006.
- Herschkovitz A, Liu YF, Ilan E, Ronen D, Boura-Halfon S, Zick Y. Common inhibitory serine sites phosphorylated by IRS-1 kinases, triggered by insulin and inducers of insulin resistance. *J Biol Chem* 282: 18018–18027, 2007.
- Hirabara SM, Silveira LR, Abdulkader F, Carvalho CR, Procopio J, Curi R. Time-dependent effects of fatty acids on skeletal muscle metabolism. J Cell Physiol 210: 7–15, 2007.
- Hiratani K, Haruta T, Tani A, Kawahara J, Usui I, Kobayashi M. Roles of mTOR and JNK in serine phosphorylation, translocation, and degradation of IRS-1. *Biochem Biophys Res Commun* 335: 836–842, 2005.
- Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 420: 333–336, 2002.
- Horike N, Takemori H, Katoh Y, Doi J, Min L, Asano T, Sun XJ, Yamamoto H, Kasayama S, Muraoka M, Nonaka Y, Okamoto M. Adipose-specific expression, phosphorylation of Ser794 in insulin receptor substrate-1, and activation in diabetic animals of salt-inducible kinase-2. J Biol Chem 278: 18440–18447, 2003.

PHOSPHORYLATION OF IRS PROTEINS

- Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH2terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes* 54, *Suppl* 2: S73–S78, 2005.
- Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259: 87–91, 1993.
- Ishibashi KI, Imamura T, Sharma PM, Huang J, Ugi S, Olefsky JM. Chronic endothelin-1 treatment leads to heterologous desensitization of insulin signaling in 3T3-L1 adipocytes. *J Clin Invest* 107: 1193–1202, 2001.
- 54. Jakobsen SN, Hardie DG, Morrice N, Tornqvist HE. 5'-AMP-activated protein kinase phosphorylates IRS-1 on Ser-789 in mouse C2C12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside. *J Biol Chem* 276: 46912–46916, 2001.
- Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444: 840–846, 2006.
- Kanzaki M, Pessin JE. Signal integration and the specificity of insulin action. *Cell Biochem Biophys* 35: 191–209, 2001.
- Karin M. The beginning of the end: IkappaB kinase (IKK) and NFkappaB activation. J Biol Chem 274: 27339–27342, 1999.
- Khan AH, Pessin JE. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia* 45: 1475– 1483, 2002.
- 59. Kim JA, Yeh DC, Ver M, Li Y, Carranza A, Conrads TP, Veenstra TD, Harrington MA, Quon MJ. Phosphorylation of Ser24 in the pleckstrin homology domain of insulin receptor substrate-1 by Mouse Pelle-like kinase/interleukin-1 receptor-associated kinase: cross-talk between inflammatory signaling and insulin signaling that may contribute to insulin resistance. *J Biol Chem* 280: 23173–23183, 2005.
- 60. Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim DW, Liu ZX, Soos TJ, Cline GW, O'Brien WR, Littman DR, Shulman GI. PKC-theta knockout mice are protected from fat-induced insulin resistance. *J Clin Invest* 114: 823–827, 2004.
- Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI. Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 108: 437–446, 2001.
- 62. Krebs M, Brunmair B, Brehm A, Artwohl M, Szendroedi J, Nowotny P, Roth E, Furnsinn C, Promintzer M, Anderwald C, Bischof M, Roden M. The Mammalian target of rapamycin pathway regulates nutrient-sensitive glucose uptake in man. *Diabetes* 56: 1600–1607, 2007.
- Krebs M, Roden M. Nutrient-induced insulin resistance in human skeletal muscle. *Curr Med Chem* 11: 901–908, 2004.
- Le Roith D, Zick Y. Recent advances in our understanding of insulin action and insulin resistance. *Diabetes Care* 24: 588–597, 2001.
- Lee S, Lynn EG, Kim JA, Quon MJ. Protein kinase C-zeta phosphorylates insulin receptor substrate-1, -3, and -4, but not -2: isoform specific determinants of specificity in insulin signaling. *Endocrinology* 149: 2451–2458, 2008.
- 66. Lee YH, Giraud J, Davis RJ, White MF. c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J Biol Chem* 278: 2896–2902, 2003.
- Leitges M, Plomann M, Standaert ML, Bandyopadhyay G, Sajan MP, Kanoh Y, Farese RV. Knockout of PKC alpha enhances insulin signaling through PI3K. *Mol Endocrinol* 16: 847–858, 2002.
- Li Y, Soos TJ, Li X, Wu J, Degennaro M, Sun X, Littman DR, Birnbaum MJ, Polakiewicz RD. Protein kinase C Theta inhibits insulin signaling by phosphorylating IRS1 at Ser(1101). J Biol Chem 279: 45304–45307, 2004.
- Liberman Z, Eldar-Finkelman H. Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. *J Biol Chem* 280: 4422–4428, 2005.
- Liu LZ, Zhao HL, Zuo J, Ho SK, Chan JC, Meng Y, Fang FD, Tong PC. Protein kinase Czeta mediates insulin-induced glucose transport through actin remodeling in L6 muscle cells. *Mol Biol Cell* 17: 2322– 2330, 2006.
- Liu YF, Herschkovitz A, Boura-Halfon S, Ronen D, Paz K, Leroith D, Zick Y. Serine phosphorylation proximal to its phosphotyrosine binding domain inhibits insulin receptor substrate 1 function and promotes insulin resistance. *Mol Cell Biol* 24: 9668–9681, 2004.
- Liu YF, Paz K, Herschkovitz A, Alt A, Tennenbaum T, Sampson SR, Ohba M, Kuroki T, LeRoith D, Zick Y. Insulin stimulates PKCzetamediated phosphorylation of insulin receptor substrate-1 (IRS-1). A

self-attenuated mechanism to negatively regulate the function of IRS proteins. *J Biol Chem* 276: 14459–14465, 2001.

- Lodhi IJ, Chiang SH, Chang L, Vollenweider D, Watson RT, Inoue M, Pessin JE, Saltiel AR. Gapex-5, a Rab31 guanine nucleotide exchange factor that regulates Glut4 trafficking in adipocytes. *Cell Metab* 5: 59–72, 2007.
- 74. Luo M, Langlais P, Yi Z, Lefort N, De Filippis EA, Hwang H, Christ-Roberts CY, Mandarino LJ. Phosphorylation of human insulin receptor substrate-1 at serine 629 plays a positive role in insulin signaling. *Endocrinology* 148: 4895–4905, 2007.
- Luo M, Reyna S, Wang L, Yi Z, Carroll C, Dong LQ, Langlais P, Weintraub ST, Mandarino LJ. Identification of insulin receptor substrate 1 serine/threonine phosphorylation sites using mass spectrometry analysis: regulatory role of serine 1223. *Endocrinology* 146: 4410–4416, 2005.
- MacAulay K, Doble BW, Patel S, Hansotia T, Sinclair EM, Drucker DJ, Nagy A, Woodgett JR. Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metab* 6: 329– 337, 2007.
- McManus EJ, Sakamoto K, Armit LJ, Ronaldson L, Shpiro N, Marquez R, Alessi DR. Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *EMBO J* 24: 1571–1583, 2005.
- Moeschel K, Beck A, Weigert C, Lammers R, Kalbacher H, Voelter W, Schleicher ED, Haring HU, Lehmann R. Protein kinase C-zetainduced phosphorylation of Ser318 in insulin receptor substrate-1 (IRS-1) attenuates the interaction with the insulin receptor and the tyrosine phosphorylation of IRS-1. *J Biol Chem* 279: 25157–25163, 2004.
- 79. Morino K, Neschen S, Bilz S, Sono S, Tsirigotis D, Reznick RM, Moore I, Nagai Y, Samuel V, Sebastian D, White M, Philbrick W, Shulman GI. Muscle specific IRS-1 Ser→Ala transgenic mice are protected from fat-induced insulin resistance in skeletal muscle. *Diabetes* 57: 2644–2651, 2008.
- Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 55, *Suppl* 2: S9–S15, 2006.
- Mothe I, Van Obberghen E. Phosphorylation of insulin receptor substrate-1 on multiple serine residues, 612, 632, 662, and 731, modulates insulin action. *J Biol Chem* 271: 11222–11227, 1996.
- Mussig K, Fiedler H, Staiger H, Weigert C, Lehmann R, Schleicher ED, Haring HU. Insulin-induced stimulation of JNK and the PI 3-kinase/mTOR pathway leads to phosphorylation of serine 318 of IRS-1 in C2C12 myotubes. *Biochem Biophys Res Commun* 335: 819–825, 2005.
- Nakamori Y, Emoto M, Fukuda N, Taguchi A, Okuya S, Tajiri M, Miyagishi M, Taira K, Wada Y, Tanizawa Y. Myosin motor Myo1c and its receptor NEMO/IKK-gamma promote TNF-alpha-induced serine307 phosphorylation of IRS-1. J Cell Biol 173: 665–671, 2006.
- Nawaratne R, Gray A, Jorgensen CH, Downes CP, Siddle K, Sethi JK. Regulation of insulin receptor substrate 1 pleckstrin homology domain by protein kinase C: role of serine 24 phosphorylation. *Mol Endocrinol* 20: 1838–1852, 2006.
- Nikoulina SE, Ciaraldi TP, Mudaliar S, Carter L, Johnson K, Henry RR. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. *Diabetes* 51: 2190–2198, 2002.
- 86. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y. A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the jux-tamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 272: 29911–29918, 1997.
- Paz K, Liu YF, Shorer H, Hemi R, LeRoith D, Quan M, Kanety H, Seger R, Zick Y. Phosphorylation of insulin receptor substrate-1 (IRS-1) by protein kinase B positively regulates IRS-1 function. *J Biol Chem* 274: 28816–28822, 1999.
- Paz K, Voliovitch H, Hadari YR, Roberts CT Jr, LeRoith D, Zick Y. Interaction between the insulin receptor and its downstream effectors. Use of individually expressed receptor domains for structure/function analysis. J Biol Chem 271: 6998–7003, 1996.
- Qiao LY, Zhande R, Jetton TL, Zhou G, Sun XJ. In vivo phosphorylation of insulin receptor substrate 1 at serine 789 by a novel serine kinase in insulin-resistant rodents. *J Biol Chem* 277: 26530–26539, 2002.

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PHOSPHORYLATION OF IRS PROTEINS

- Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C-zeta phosphorylates insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in response to insulin. J Biol Chem 276: 3543–3549, 2001.
- 91. Rosenzweig T, Braiman L, Bak A, Alt A, Kuroki T, Sampson SR. Differential effects of tumor necrosis factor-alpha on protein kinase C isoforms alpha and delta mediate inhibition of insulin receptor signaling. *Diabetes* 51: 1921–1930, 2002.
- Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799–806, 2001.
- Sampson SR, Cooper DR. Specific protein kinase C isoforms as transducers and modulators of insulin signaling. *Mol Genet Metab* 89: 32–47, 2006.
- Sawka-Verhelle D, Baron V, Mothe I, Filloux C, White MF, Van Obberghen E. Tyr624 and Tyr628 in insulin receptor substrate-2 mediate its association with the insulin receptor. *J Biol Chem* 272: 16414– 16420, 1997.
- Sawka-Verhelle D, Tartare-Deckert S, White MF, Van Obberghen E. Insulin receptor substrate-2 binds to the insulin receptor through its phosphotyrosine-binding domain and through a newly identified domain comprising amino acids 591–786. J Biol Chem 271: 5980–5983, 1996.
- 96. Serra C, Federici M, Buongiorno A, Senni MI, Morelli S, Segratella E, Pascuccio M, Tiveron C, Mattei E, Tatangelo L, Lauro R, Molinaro M, Giaccari A, Bouche M. Transgenic mice with dominant negative PKC-theta in skeletal muscle: a new model of insulin resistance and obesity. J Cell Physiol 196: 89–97, 2003.
- 97. Sharfi H, Eldar-Finkelman H. Sequential phosphorylation of insulin receptor substrate-2 by glycogen synthase kinase-3 and c-Jun NH₂terminal kinase plays a role in hepatic insulin signaling. *Am J Physiol Endocrinol Metab* 294: E307–E315, 2008.
- Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 116: 1793–1801, 2006.
- Solinas G, Naugler W, Galimi F, Lee MS, Karin M. Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc Natl Acad Sci USA* 103: 16454–16459, 2006.
- Sommerfeld MR, Metzger S, Stosik M, Tennagels N, Eckel J. In vitro phosphorylation of insulin receptor substrate 1 by protein kinase C-zeta: functional analysis and identification of novel phosphorylation sites. *Biochemistry* 43: 5888–5901, 2004.
- 101. Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, Annes J, Petrzilka D, Kupfer A, Schwartzberg PL, Littman DR. PKC-theta is required for TCR-induced NF-kappaB activation in mature but not immature T lymphocytes. *Nature* 404: 402–407, 2000.
- 102. Tremblay F, Brule S, Hee Um S, Li Y, Masuda K, Roden M, Sun XJ, Krebs M, Polakiewicz RD, Thomas G, Marette A. Identification of IRS-1 Ser-1101 as a target of S6K1 in nutrient- and obesity-induced insulin resistance. *Proc Natl Acad Sci USA* 104: 14056–14061, 2007.

- 103. Tzatsos A, Tsichlis PN. Energy depletion inhibits phosphatidylinositol 3-kinase/Akt signaling and induces apoptosis via AMP-activated protein kinase-dependent phosphorylation of IRS-1 at Ser-794. *J Biol Chem* 282: 18069–18082, 2007.
- Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. *Cell Metab* 3: 393–402, 2006.
- 105. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 431: 200–205, 2004.
- 106. Usui I, Imamura T, Babendure JL, Satoh H, Lu JC, Hupfeld CJ, Olefsky JM. G protein-coupled receptor kinase 2 mediates endothelin-1-induced insulin resistance via the inhibition of both Galphaq/11 and insulin receptor substrate-1 pathways in 3T3-L1 adipocytes. *Mol Endocrinol* 19: 2760–2768, 2005.
- 107. Voliovitch H, Schindler DG, Hadari YR, Taylor SI, Accili D, Zick Y. Tyrosine phosphorylation of insulin receptor substrate-1 in vivo depends upon the presence of its pleckstrin homology region. *J Biol Chem* 270: 18083–18087, 1995.
- Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, Klip A. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol Cell Biol* 19: 4008–4018, 1999.
- Wellen KE, Hotamisligil GS. Inflammation, stress, diabetes. J Clin Invest 115: 1111–1119, 2005.
- Wu J, Tseng YD, Xu CF, Neubert TA, White MF, Hubbard SR. Structural and biochemical characterization of the KRLB region in insulin receptor substrate-2. *Nat Struct Mol Biol* 15: 251–258, 2008.
- 111. Yi Z, Langlais P, De Filippis EA, Luo M, Flynn CR, Schroeder S, Weintraub ST, Mapes R, Mandarino LJ. Global assessment of regulation of phosphorylation of insulin receptor substrate-1 by insulin in vivo in human muscle. *Diabetes* 56: 1508–1516, 2007.
- Youngren JF. Regulation of insulin receptor function. *Cell Mol Life Sci* 64: 873–891, 2007.
- 113. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, Cushman SW, Cooney GJ, Atcheson B, White MF, Kraegen EW, Shulman GI. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem 277: 50230–50236, 2002.
- 114. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673– 1677, 2001.
- Zick Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. *Trends Cell Biol* 11: 437–441, 2001.
- Zick Y. Molecular basis of insulin action. Novartis Found Symp 262: 36–50, disucssion 50–35, 265–268, 2004.
- Zick Y. Ser/Thr phosphorylation of IRS proteins: a molecular basis for insulin resistance. *Sci STKE* 2005: pe4, 2005.