Fatty acid-induced insulin resistance: role of insulin receptor substrate 1 serine phosphorylation in the retroregulation of insulin signalling

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Abstract
Insulin resistance, when combined with impaired insulin secretion, contributes to the development of type 2 diabetes. Insulin resistance is characterized by a decrease in the insulin effect on glucose transport in muscle and adipose tissue. Tyrosine phosphorylation of IRS-1 (insulin receptor substrate 1) and its binding to PI 3-kinase (phosphoinositide 3-kinase) are critical events in the insulin signalling cascade leading to insulin-stimulated glucose transport. Various studies have implicated lipids as a cause of insulin resistance in muscle. Elevated plasma fatty acid concentrations are associated with reduced insulin-stimulated glucose transport activity as a consequence of altered insulin signalling through PI 3-kinase. Modification of IRS-1 by serine phosphorylation could be one of the mechanisms leading to a decrease in IRS-1 tyrosine phosphorylation, PI 3-kinase activity and glucose transport. Recent findings demonstrate that non-esterified fatty acids, as well as other factors such as tumour necrosis factor α, hyperinsulinaemia and cellular stress, increase the serine phosphorylation of IRS-1 and identified Ser307 as one of the phosphorylated sites. Moreover, several kinases able to phosphorylate this serine residue have been identified. These exciting results suggest that Ser307 phosphorylation is a possible hallmark of insulin resistance in biologically insulin-responsive cells or tissues. Identification of IRS-1 kinases could enable rational drug design in order to selectively inhibit the activity of the relevant enzymes and generate a novel class of therapeutic agents for type 2 diabetes.

Introduction: insulin action and insulin resistance
Insulin is the primary hormone involved in glucose homoeostasis and in the stimulation of glucose transport. Circulating insulin rapidly reaches its target tissues (mainly liver, muscles and adipose tissue), where it interacts with its receptor. The IR (insulin receptor) belongs to the family of cell-surface receptors possessing intrinsic tyrosine kinase activity. IR is composed of two extracellular α-subunits and two transmembrane β-subunits linked by disulphide bonds. Insulin binds to the α-subunit to promote tyrosine autophosphorylation of the β-subunit of the IR. The activated IR phosphorylates its substrates, including the IRS (insulin receptor substrate) proteins, Shc (Src homology collagen) and APS [adapter protein with a PH (pleckstrin homology) and SH2 (Src homology 2) domain]. The phosphorylated proteins dock downstream effector molecules which activate different signalling pathways. The MAPK (mitogen-activated protein kinase) pathway mediates the growth-promoting function of insulin. The PI 3-kinase (phosphoinositide 3-kinase) and the TC10 pathways are involved in the metabolic actions of insulin such as an increase in glucose transport (Figure 1) [1].

Insulin resistance is a common pathological state in which target tissues fail to respond properly to normal levels of circulating insulin. Pancreatic β-cells first compensate for peripheral insulin resistance by increasing insulin secretion to maintain euglycaemia. Thereafter, impaired glucose tolerance can develop, leading to overt clinical type 2 diabetes. Non-esterified fatty acids (NEFA) play an important role in the establishment of insulin resistance [2]. Indeed, chronic elevation in plasma NEFA levels is commonly associated with impaired insulin-mediated glucose uptake in skeletal muscles and often coexists with obesity and type 2 diabetes. In the 1960s, Randle and co-workers proposed a mechanism for fat-induced insulin resistance, which implicated fatty acid oxidation as the cause of this defect [3,4]. The use of novel technology such as NMR spectroscopy allowed a more direct study of glucose metabolism in vivo, and led Shulman and others to propose the glucose transport itself as being responsible for the observed defects [2,5]. At the molecular level, a decrease in glucose uptake is linked to reduced tyrosine phosphorylation of IRS-1 and PI 3-kinase activation. Although the mechanisms involved in the decrease in IRS-1 tyrosine phosphorylation are not completely identified, the role of the Ser phosphorylation of IRS-1 in the desensitization

Key words: free (non-esterified) fatty acid, mammalian target of rapamycin (mTOR), muscle insulin resistance, serine phosphorylation, tumour necrosis factor α (TNFα).

Abbreviations used: IRS, insulin receptor substrate; PI 3-kinase, phosphoinositide 3-kinase; IRS, tumour necrosis factor; NEFA, non-esterified fatty acids; IR, insulin receptor; mTOR, mammalian target of rapamycin; I KK, β, inhibitor of κB kinase β; PH, pleckstrin homology; PKC, protein kinase C; PP1, protein kinase C; SH2, Src homology collagen; MAPK, mitogen-activated protein kinase; JNK, c-jun N-terminal kinase.

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Insulin interacts with the α-subunits of its receptor, leading to an increase in the autophosphorylation and the tyrosine kinase activity of the β-subunits. The docking proteins Shc and IRS-1 interact with the Tyr960 in the juxtamembrane domain of the receptor while APS (adapter protein with a PH and SH2 domain) interacts with Tyr1158/1162 in the catalytic domain. Those interactions allow the tyrosine phosphorylation (pY) of these proteins which in turn bind and activate several signalling proteins. Recruitment of the Grb2–SOS complex to tyrosine-phosphorylated Shc activates the MAPK cascade, which is involved in cell growth and regulation of gene expression. Association between PI 3-kinase (PI3K) and IRS-1 increases the amount of PtdIns3P that activates PDK1 and its effectors, the Ser/Thr kinases PKB and atypical PKCζ. These kinases are involved in the stimulation of glucose uptake. PKB is also involved in insulin-induced glycogen synthesis by phosphorylating and inactivating glycogen synthase kinase-3. The interaction between tyrosine-phosphorylated APS and the CAP–Cbl complex allows the tyrosine phosphorylation of Cbl by the insulin receptor. Phosphorylation of Cbl mediates glucose transport by a pathway independent of PI 3-kinase and dependent on the activation of the small GTPase TC10. MEK, MAPK/extracellular-signal-regulated kinase kinase. Sos, son of sevenless; PDK1, 3-phosphoinositide-dependent protein kinase-1; CAP, Cbl-associated protein; C3G, Crk SH3 binding guanine-nucleotide releasing factor.

Role of fatty acids in glucose metabolism: Randle’s hypothesis

Several results favour a role of NEFA in insulin resistance. Indeed, fasting and postprandial NEFA concentrations are elevated in both diabetes and in individuals at risk for the disease. An inverse relationship between fasting plasma NEFA concentration and insulin sensitivity has been observed in young, normal-weight offspring of type 2 diabetic patients [6]. Muscle insulin resistance can be induced by infusion of lipid emulsion with heparin to acutely raise plasma fatty acid concentrations (for review of the literature, see [4]). Finally, strong evidence about a role of lipids in muscle insulin resistance has been obtained from studies showing that the intramyocellular lipid content was inversely correlated with insulin sensitivity in the same group of subjects [2].

Randle proposed the following mechanism for lipid-induced insulin resistance: NEFA compete with glucose for substrate oxidation and increased fat oxidation may cause the insulin resistance in obesity and type 2 diabetes. According to this hypothesis, increased NEFA levels lead to elevated mitochondrial acetyl-CoA/CoA and NADH/NAD ratios which, in turn, inhibit pyruvate dehydrogenase activity and lead to an increase in citrate levels that inhibits phosphofructokinase activity. This process induces an increase in glucose 6-phosphate concentration, which inhibits hexokinase and reduces glucose transport/phosphorylation activity. A detailed review of this hypothesis has been published 35 years after its first proposal [4].

Role of fatty acids in glucose metabolism: Shulman’s hypothesis

Shulman et al. [2,7] challenged the conventional Randle hypothesis with an elegant series of studies. Using NMR spectroscopy, they demonstrated that an elevation of the
plasma fatty acid concentrations results in a decrease rather than the expected increase in the concentration of glucose 6-phosphate in muscle. The initial defect appears to be an alteration in glucose transport in response to insulin [2,7].

Shulman and colleagues indeed demonstrated that an elevation in plasma NEFA concentrations for 5 h induced insulin resistance as reflected by a reduction in glucose infusion rate during hyperinsulinaemic–euglycaemic clamp. Insulin-stimulated rates of muscle glycogen synthesis and whole-body glucose oxidation were altered compared with controls. This effect of NEFA appears very rapidly [8]. The observed fall in intramuscular glucose 6-phosphate reflected an inhibition of glucose transport and/or phosphorylation. Further experiments were in favour of a defect in glucose transport itself, which was associated with a strong decrease in IRS-1 tyrosine phosphorylation and PI 3-kinase activation. Based on these data, Shulman and colleagues [2] proposed that fatty acids cause insulin resistance through inhibition of IRS-1 signalling.

IRS-1 serine phosphorylation and cellular insulin resistance

The concept that IRS-1 Ser phosphorylation impairs insulin action emerged 10 years ago when Tanti et al. [9,10] showed that a Ser phosphatase inhibitor, okadaic acid, severely altered the effect of insulin on glucose transport and Glut 4 translocation in adipocytes and skeletal muscles. This effect was linked to a decrease in IRS-1 tyrosine phosphorylation and PI 3-kinase activation. We found that okadaic acid markedly increases the Ser phosphorylation of IRS-1 and that hyperphosphorylated IRS-1 is a poor substrate for the IR [10]. The same process occurred in cells treated with platelet-derived growth factor [11,12]. Interestingly, a prolonged stimulation of cells with insulin also induces the Ser phosphorylation of IRS-1 suggesting that it could be a negative-feedback mechanism that uncouples the IRS-1 proteins from their upstream and downstream partners and blocks insulin signal transduction under physiological conditions. Emerging data demonstrate that several factors implicated in insulin resistance use this process to down-regulate insulin signalling. Thus, Ser phosphorylation of IRS-1 represents a new and possibly unifying mechanistic process involved in insulin resistance. Identification of the involved kinases is thus challenging.

Serine phosphorylation of IRS-1 induced by TNFα, insulin and hyperosmotic stress

The proinflammatory cytokine TNFα has been implicated as a link between obesity and insulin resistance although its role in humans is still controversial. TNFα increases the serine phosphorylation of IRS-1 which inhibits insulin-stimulated tyrosine phosphorylation and impairs insulin signalling. Recently, phosphopeptide mapping, mutational analysis and phosphospecific antibodies allowed the identification of the serine residues phosphorylated in response to insulin and TNFα. Interestingly, insulin and TNFα result in phosphorylation of the same residues in IRS-1, namely Ser307, Ser612 and Ser632 [13–16] (Figure 2). These observations suggest that TNFα inhibits insulin signalling by taking advantage of the physiological mechanism used by insulin to terminate its signal transduction.

Ser307 is located at the end of the phosphotyrosine-binding domain involved in the interaction of IRS-1 with IR. While its phosphorylation blocks this interaction [17,18], its mutation prevents the inhibitory effect of TNFα on insulin-induced IRS-1 tyrosine phosphorylation. Ser307 phosphorylation could induce a conformational change of the phosphotyrosine-binding domain that reduces its affinity for the IR [17,18]. The use of kinase inhibitors or of knockout mice indicated that both JNK (c-Jun N-terminal kinase) and IKK-β (inhibitor κB kinase β) are involved in Ser307 phosphorylation [16,19–22]. Moreover, activation of both kinases is required for full phosphorylation of this site by TNFα [16]. While insulin also induces the phosphorylation of Ser307 in IRS-1, the kinase involved is different. Indeed, in agreement with [23], we found that the mTOR (mammalian target of rapamycin) signalling pathway was predominantly involved in insulin-induced phosphorylation of Ser307 in adipocytes, muscles and hepatocytes [14], although JNK could also be involved [21]. Hyperosmotic stress, which also induces insulin resistance, increases the phosphorylation of IRS-1 on Ser307 by an mTOR-dependent pathway [24,25].

Ser612 and Ser632 are located close to tyrosine residues which are major phosphorylation sites involved in the binding of PI 3-kinase and are required for insulin-stimulated glucose uptake. The role of the phosphorylation of these two serine residues is not firmly established but several studies suggest that it could modulate the interaction between IRS-1 and PI 3-kinase and/or its activation [26,27]. Phosphorylation of these sites is mediated by MAPK and/or mTOR signalling pathways in response to both insulin [14] and TNFα [15,16]. Interestingly, we recently reported that the basal level of IRS-1 phosphorylation on Ser632 was abnormally high in primary culture of skeletal muscle cells obtained from type 2 diabetic patients [28]. Concomitantly, the basal activity of MAPK was increased and insulin-induced IRS-1 tyrosine phosphorylation was altered. Moreover, inhibition of MAPK normalized the level of IRS-1 Ser632 phosphorylation [28]. While it remains to be determined whether such normalization reverses the defect in insulin action, these results favour a role of MAPK in the decrease in insulin sensitivity in type 2 diabetic patients.

Serine phosphorylation of IRS-1 induced by NEFA

A similar process appears to be induced by NEFA. Shulman and collaborators demonstrated that the decrease in insulin-induced IRS-1 tyrosine phosphorylation provoked by NEFA was linked to an increase in the activity of protein kinase C (PKC) θ [29]. PKCθ activity is increased by diacylglycerol.
produced by the metabolism of muscle long-chain acyl-CoA. Activation of PKCθ induces the Ser³⁰⁷ phosphorylation of IRS-1 that could explain the decrease in insulin-induced IRS-1 tyrosine phosphorylation [30]. Interestingly, high-fat feeding also increases muscle long-chain acyl-CoA content, PKCθ activity and Ser³⁰⁷ phosphorylation [2].

IKK-β that is activated by PKCθ could be involved in the phosphorylation of IRS-1 in response to high NEFA concentrations. Indeed, a high dose of salicylate, which improves glucose tolerance and inhibits IKK-β, prevented muscle insulin resistance and the decrease in IRS-1 tyrosine phosphorylation induced by lipid infusion [31]. Moreover, lipid infusion failed to alter insulin signalling in skeletal muscle of IKK-β heterozygous knockout mice, which are also protected against high-fat diet-induced insulin resistance and the decrease in IRS-1 tyrosine sensitivity [32].

JNK could also be involved in the phosphorylation of IRS-1 in fat-induced insulin resistance. Indeed, JNK is activated by NEFA and JNK activity is abnormally elevated in obesity [19]. Interestingly, JNK1-knockout mice have a decreased adiposity, resistance to a high-fat diet, an improved insulin sensitivity and an enhanced insulin receptor signalling capacity. Moreover, genetically obese (ob/ob) mice with a targeted mutation in Jnk1 put on less weight than their relative control (ab/ab) mice and they are partly protected against hyperinsulinaemia and hyperglycaemia [19]. Thus, selective interference with JNK1 or with IKK-β could present an attractive opportunity for the treatment of human obesity, insulin resistance and type 2 diabetes.

In conclusion, serine phosphorylation of IRS-1 seems to be a very complex process which is indicative of its important physiological relevance [22]. Many sites have been identified and various kinases (MAPK, JNK, IKK-β, mTOR) are involved. Phosphorylation of Ser⁶¹²/Ser⁶³² in addition to the closely located tyrosine residues could accurately regulate PI 3-kinase activity. Phosphorylation of Ser³⁰⁷ could have a more general role in the regulation of insulin sensitivity. Its phosphorylation, by inhibiting the interaction between the IR and IRS-1, could favour the dephosphorylation of all IRS-1 tyrosine phosphorylation sites, leading to termination of the insulin signal. Moreover, the regulation of serine versus tyrosine phosphorylation of IRS-1 may regulate IRS-1 degradation, since IRS-1 with a point mutation of Ser³⁰⁷ is more resistant to degradation following long-term exposure to insulin [23].

**Fatty acids and Ser/Thr phosphatase activation**

Apart from an increase in serine kinase activity against IRS-1, other mechanisms have also been proposed to explain the
NEFA-induced insulin resistance. It has been demonstrated that exposing muscle cells to saturated NEFA inhibits insulin stimulation of protein kinase B (PKB), a serine kinase downstream of PI 3-kinase that is involved in insulin-induced glucose uptake. This inhibition has been linked with an increase in ceramide content from fatty acyl-CoA metabolism. Ceramide could activate a phosphatase, namely protein phosphatase 2A, which is involved in the dephosphorylation and deactivation of Akt/PKB and thus, by maintaining PKB in an inactive state, can participate in fat-induced muscle insulin resistance [33].

Conclusion
In conclusion, it is clear that an increase in fatty acids within the muscle cells often leads to insulin resistance. Thus overexpression of lipoprotein lipase, either in liver or in muscle, led to tissue-specific insulin resistance [34]. It should however be observed that a high level of triglyceride (triacylglycerol) in muscle is not always linked to insulin resistance. Indeed, exercise training increases muscle triglyceride content with the capacity of the muscle for fatty acid metabolism. Thus overexpression of lipoprotein lipase, either in liver or in muscle, led to tissue-specific insulin resistance [34].

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