

Insulin Action in Peripheral Glucose Uptake - The Molecular Perspective

Acção Periférica da Insulina na Captação de Glucose – a Perspectiva Molecular

Ricardo A. Afonso, PhD^{1,2}

¹ CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa

² Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve

Correspondência: Ricardo A. Afonso › Área de Ensino e Investigação em Ciências Funcionais e Alvos Terapêuticos (Departamento de Fisiologia) › Faculdade de Ciências Médicas › Universidade Nova de Lisboa › Campo Mártires da Pátria, 130, 1169-056 LISBOA › ricardo.afonso@fcm.unl.pt

RESUMO

Nos últimos anos, a insulinoresistência tem sido objecto de inúmeros estudos e um dos principais alvos da pesquisa e intervenção farmacológicas. Como consequência, importantes passos têm sido dados a um ritmo acelerado, com vista à compreensão dos mecanismos associados à acção da insulina, bem como às suas alterações. Deste modo, a tomada de conhecimento dos mais recentes avanços nesta área e de como eles se encaixam na panorâmica global da acção da insulina parece ser útil, tanto do ponto de vista da pesquisa como do ponto de vista clínico.

O presente é o primeiro de dois mini-artigos de revisão acerca da acção da insulina no aporte periférico de glucose. Esta primeira revisão tem como objectivo dar uma visão geral dos eventos intracelulares conducentes à captação de glucose insulino-dependente, enquanto que na segunda revisão será efectuada uma abordagem da acção da insulina numa perspectiva fisiológica, *ie*, integrativa, dando particular ênfase às diferenças na acção da insulina de acordo com o estado prandial.

Assim, na presente publicação será dada uma visão sumária e geral das principais vias de transdução de sinal da insulina, envolvidas no aporte de glucose por tecidos periféricos (extra-hepáticos). Apesar de neste artigo não se fazer uma abordagem farmacológica, espera-se que constitua uma boa base para compreender os mecanismos associados à fisiopatologia e farmacologia das alterações na acção da insulina.

PALAVRAS-CHAVE

Insulina; Acção da insulina; Receptor de insulina; Transdução de sinal da insulina; Aporte de glucose.

ABSTRACT

In the recent years, insulin resistance has become the aim of numerous studies and one of the major focuses for pharmacological research and intervention. As a logical consequence, important steps towards the knowledge of insulin action and its alterations have been added at a high rate. Therefore, the awareness about the recent breakthroughs in this field and about how they fit within the whole picture of insulin action seems to be very useful, both in clinical and research practice. The present article is the first of two mini-reviews concerning peripheral insulin action in glucose uptake. This first review article aims at the intracellular events leading to insulin-dependent glu-

cose uptake, whereas in the second review insulin action will be approached in a whole-body perspective, giving particular emphasis to differences in insulin action according to the prandial state. Thus, in the present review, we will provide a brief overview of the major insulin signaling pathways involved in peripheral (extra-hepatic) glucose uptake. Although this article does not aim pharmacological therapeutic, we hope that it may launch some minimum comprehensive basis to better understand the mechanism behind the pathophysiology and pharmacology of insulin action.

KEYWORDS

Insulin; Insulin action; Insulin receptor; Insulin signalling pathway; Glucose uptake.

INTRODUCTION

Insulin is probably the most important anabolic hormone in the human organism¹. At the cellular level, its action is characterized by several effects, which suggests the involvement of multiple signaling pathways initiated by the binding of insulin to the receptor.

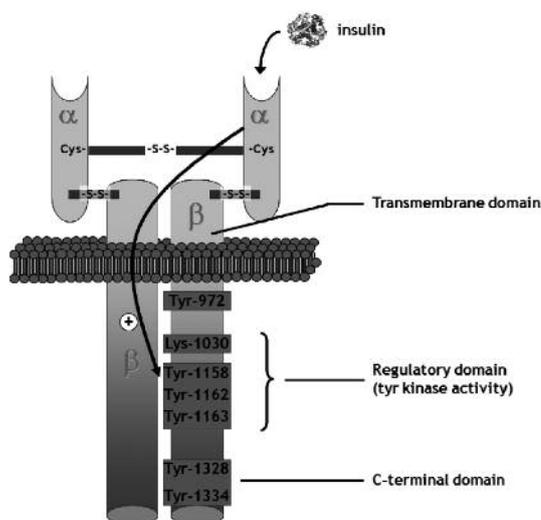
The present review aims to provide a brief overview of the major insulin signaling pathways involved in glucose uptake via GLUT4 translocation, in particular in adipose tissue and skeletal muscle, last of which is responsible for about 75 % of the insulin-dependent glucose uptake². Transposition from the cellular to the physiological level (*ie*, whole-body) will be essayed in a second review, resulting in a broad outline of insulin action on glucose metabolism and on glucose uptake in particular.

INSULIN RECEPTOR

The insulin receptor is ubiquitous in vertebrate tissues, although it may be expressed in different concentrations in different tissues³. A general schematic representation of the insulin receptor is provided in figure 1.

Structurally, insulin receptor is an heterotetrameric glycoprotein, composed of two α -subunits and by two β -subunits, with N-terminal complex carbohydrates capped by terminal sialic acid residues^{4,5}. Insulin receptor structure is stabilized by 3 disulphide

FIGURE 1



Schematic representation of the insulin receptor. The insulin receptor presents two extracellular α -subunits, which contain the domains of insulin binding, and two β -subunits, where occurs binding of ATP and tyrosine phosphorylation (regulatory domain – intracellular portion of β -subunits). Cys, cysteine residue; -S-S-, disulphide bond; Tyr, tyrosine residue; Lys, lysine residue. ⊕, activation.

bonds that link the two α -subunits to each other and to the β -subunits, presenting a $(\alpha\beta)_2$ organization^{6,7}. The α -subunits are entirely located in the outside the cell, whereas β -subunits contain one extracellular portion, one transmembrane region and an intracellular region, last of which includes a juxtamembrane domain, a regulatory domain (activation domain) and a C-terminal domain^{6,8}, with different functional roles.

Presently, there are two types of insulin receptor described: types A and B. The difference between these two isoforms is the presence of a 12 aminoacid sequence between

positions 716 and 717 of the α -subunits of type A insulin receptor⁹ Type B insulin receptor is highly specific for insulin and prominent in the major target-tissues for insulin action, such as liver, skeletal muscle and adipose tissue¹⁰. Type A insulin receptor promotes binding of IGF-2 (insulin-like growth factor 2) instead of insulin and is present in many fetal tissues, central nervous system and haematopoietic cells¹⁰. Patients with accumulation of type A receptor in skeletal muscle seem to be more prompt to the development of insulin resistance¹¹.

INSULIN BINDING AND ACTIVATION OF THE RECEPTOR

Insulin binds to one of the α -subunits of the insulin receptor, bringing the two α -subunits closer upon disruption of the α_2 -dimer^{6,7}. Although there are two major binding sites (in the two α -subunits – figure 1), only one insulin molecule binds to the insulin receptor with high affinity, presenting a negative cooperativity for insulin concentrations lower than 0.1 $\mu\text{mol}/\text{dm}^3$ ¹².

Insulin binding to the α -subunit induces tyrosine kinase activity in the regulatory domain of the intracellular portion of the β -subunit, promoting phosphorylation of tyrosine residues of this domain and concomitant activation of the insulin receptor - autophosphorylation.

Autophosphorylation of the insulin receptor is the key step in the initiation of the intracellular signalling and it may occur at seven different tyrosine residues, located in the three regions of the β -subunits with tyrosine kinase activity (juxtamembrane, regulatory or activation and C-terminal)³. However, the process seems to be initiated by phosphorylation of the tyrosine¹¹⁶² residue of the regulatory domain⁶ (figure 1).

Insulin binding induces conformational changes in the regulatory (or activation) domain that allow binding to ATP, favoring the initial phosphorylation of the tyro-

sine¹¹⁶² residue (regulatory domain) and, subsequently, the remaining tyrosine residues of the regulatory domain of the insulin receptor⁶. Phosphorylation of tyrosine residues in the insulin receptor allow the recruitment, docking and activation of the effector proteins involved in the signalling cascade that present SH2 (Src-2 homology) domains^{2,13,14}. Many of these effector proteins are small adaptive molecules, such as p85, which is the regulatory subunit of the enzyme phosphatidylinositol-3-kinase (PI3K) and of CrkII, a small protein G activation molecule².

After insulin binding and activation of the insulin receptor, the complex insulin-insulin receptor is internalized and incorporated into endosomes, still in an active form, which facilitates the binding of the cytoplasmatic substrates¹⁵.

Interestingly, in the absence of insulin, α -subunits seem to exert a negative effect upon the regulatory domains, thus blocking the signal transduction cascade^{2,3,6,16,17}.

This unusual form of activation seems to allow small molecules to interact with the insulin receptor in distinct sites from the activation domains of insulin¹⁸.

INSULIN SIGNALING PATHWAYS INVOLVED IN GLUCOSE UPTAKE

Both insulin receptor and the majority of the proteins involved in insulin signalling are activated by tyrosine residues phosphorylation¹³.

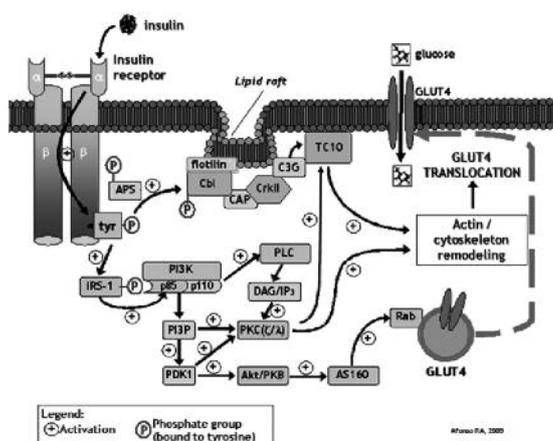
There are several intracellular substrates of the insulin receptor that can be phosphorylated at tyrosine residues by the receptor itself: Gab1, p60^{shc}, APS, Shc isoforms, Cbl and the proteins of the IRS family (insulin receptor substrate). Many of these proteins are common substrates of the insulin receptor and of the IGF-1 receptor; however, the different specificity of both recruitment and phosphorylation ensure an adequate regulation of the signalling cascades of insulin

and IGF-1 receptors¹⁹.

Additionally, APS, Cbl and IRS proteins, in particular, have been associated with the process of glucose uptake through stimulation of glucose transporters-4 (GLUT4) translocation².

Figure 2 summarizes the major insulin signaling pathways that involve these substrates, leading to GLUT4 translocation and glucose uptake.

FIGURE 2



Major insulin signaling pathways for glucose uptake acting through glucose transporters-4 (GLUT4). Insulin receptor substrate-1 (IRS1) was used, since it is the most common protein in the IRS family. Tyr, tyrosine; PI3K, 3-phosphatidylinositol kinase; PI3P, 3-phosphatidylinositol phosphate; PDK1, phosphoinositide-dependent kinase; Akt/PKB, protein B kinase; PKC ζ/λ , atypical protein C kinases; AS160, Akt Substrate of 160 kDa; PLC, phospholipase C; DAG, diacylglycerol; IP₃, 1,4,5-inositol triphosphate; APS, adaptative protein with PH and SH2 domains; Cbl, protooncogene Casitas b-lineage lymphoma (c-Cbl); CAP, Cbl-associated protein.

The most relevant mediators of insulin action in glucose uptake by skeletal muscle and adipocytes are the IRS proteins, in particular IRS-1 and IRS-2. In mammals, four major proteins of the IRS family were described: IRS-1, expressed in skeletal muscle and adipose tissue; IRS-2, present in the brain, ovary, liver and adipose tissue; IRS-3, expressed in adipose tissue, presumably in rodents only; and IRS-4, present in the thymus and kidney¹³. IRS proteins present an amine terminal, with binding domains for the insulin receptor and a carboxyl terminal, with tyrosine phosphorylation sites²⁰.

Following tyrosine phosphorylation, IRS

protein activates PI3K, which plays a central role in GLUT4 translocation. IRS activates PI3K by binding to p85 regulatory subunit, which presents two SH2 domains that bind to phosphorylated residues in IRS proteins². Besides p85 subunit, PI3K presents a p110 catalytic subunit, responsible for phosphoinositides phosphorylation at position 3, producing phospholipidic compounds of the phosphatidylinositol-3-phosphate (PI3P) family, namely phosphatidylinositol-3,4,5-triphosphate (PtdInsP₃)².

PI3P (and PtdInsP₃ in particular) activate phosphoinositide-dependent kinase 1 (PDK1), which in turn activates protein kinase B (Akt/PKB) and the atypical protein kinase C (PKC ζ and PKC λ)². It has also been described that PtdInsP₃ can bind directly to PKC (PKC ζ and PKC λ)¹³ and to Akt/PKB, activating them², therefore not requiring PDK1 as an intermediate.

Active Akt/PKB then promotes phosphorylation of Akt Substrate of 160 kDa protein (AS160)²¹, which is constitutively associated to GLUT4 vesicles²² and in particular to Rab proteins, small G proteins involved in the processes of transport and fusion of GLUT4 vesicles to plasma membrane²³. Thus, AS160 phosphorylation by Akt/PKB promotes activation of the Rab proteins^{22,24}, leading to a higher rate of GLUT4 translocation²³ - this topic will be further explored in the next section. On the other hand, PI3K can activate phospholipase C (PLC), resulting in the production of the second messengers DAG and inositol triphosphate (IP₃), which activate PKC ζ , thus stimulating glucose uptake²² (figure 2).

An additional insulin signalling pathway contributing to GLUT4 translocation and somehow independent of IRS phosphorylation and PI3K activation is described¹, and also presented in figure 2.

Such pathway involves phosphorylation of both APS (adaptive protein with SH2 and PH domains, last of which is present in Akt/PKB, allowing this enzyme to bind

PtdInsP₃) and Cbl protooncogene (Casitas b-lineage lymphoma, c-Cbl)^{2,25} directly by the IR^{1,2,25}. APS is involved in Cbl recruitment for the insulin receptor²⁶. In the majority of insulin-sensitive cells Cbl is associated with the adaptive protein CAP (Cbl-associated protein)². Following phosphorylation, the Cbl-CAP complex is transported into lipid rafts in the plasma membrane, where it binds to flotilin and recruits CrkII protein². CrkII then forms a complex with the guanyl nucleotide exchange protein C3G²⁷, which activates TC10^{22,28}. TC10 is a GTP-binding protein present in the lipid rafts that contributes to GLUT4 translocation and their docking at the plasma membrane^{26,28}, possibly through regulation of actin microfilaments dynamics^{22,29,30}.

Although the TC10 pathway can be seen as an separate pathway from the PI3K-dependent one, some studies have suggested that TC10 activates PI3P³¹ and others have described that atypical PKC (PKC ζ and PKC λ) are also able to promote TC10 activation^{26,32}. Thus, atypical PKC may represent a point of convergence for the PI3K and TC10 signaling pathways¹⁹, both of which contributing synergistically to GLUT4 translocation (figure 2).

GLUT4 TRANSLOCATION

GLUT4, present mostly in skeletal muscle and adipocyte, are located within vesicles that move in a cyclic manner between the intracellular storing sites and plasma membrane. Insulin promotes the presence of GLUT4 at the plasma membrane in two distinct, but synergistic ways: by increasing the rate of GLUT4 exocytosis and by reducing their internalization rate^{2,33}.

As in the case of the insulin secretory granules in β -pancreatic cells, GLUT4 vesicles also seem to be translocated towards the plasma membrane by means of a system involving microtubules network and actin polymerization^{2,22}. Actin remodeling is

required not only for translocation of the GLUT4 vesicles, but also to their fusion with the plasma membrane².

As stated in the previous section and presented in figure 2, the remodeling or reorganization of the actin filaments in response to insulin binding to the receptor appears to be modulated by both the TC10 and IRS/PI3K pathways, through activation of the Rab proteins²².

Rab proteins have been shown to be necessary effectors in vesicle trafficking, docking and fusion. In particular, Rabs 2A, 8A, 10, and 14 are expressed in insulin-sensitive tissues and appear to be substrates of the AS160 GAP domain (IRS/PI3K pathway) and are associated with insulin-responsive GLUT4-containing vesicles³⁴⁻³⁶. AS160 thus may represent a convergence between insulin signaling and vesicle trafficking²². AS160 is a negative regulator of basal GLUT4 exocytosis, ie, in basal conditions, AS160 associates with GLUT4 vesicles, maintaining Rab proteins in their inactive form (Rab-GDP)^{33,34}. Insulin-stimulated phosphorylation of S160 (PI3K pathway) inhibits AS160 negative effect on Rab proteins, causing a shift towards Rabs activation (Rab-GTP complex formation) and allowing for Rab-dependent GLUT4 translocation to occur^{33,34,37}.

As mentioned earlier, TC10 can also stimulate the Rab proteins mechanism through activation of PI3P (figure 2). Additionally, TC10 seems to activate actin-related protein 3 (Arp3), actin-regulatory neural Wiskott-Aldrich syndrome protein (N-WASP)³⁰ and exocyst protein complex³⁸, which are involved in the regulation of actin polymerization (N-WASP and Arp3), as well as docking and anchoring of GLUT4 vesicles to the plasma membrane (exocyst protein complex)^{22,30,38}. This TC10-mediated process is required for the subsequent fusion of GLUT4 vesicles to the plasma membrane carried out by soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein

receptors (SNARE), namely SNAP-23, syntaxin 4, Synip, Munc18c and vesicle-associated membrane protein-2 (VAMP2) and and the plasma membrane proteins synaptosome-associated 25-kDa protein and syntaxin-1A^{22,39-41}.

INHIBITION OF THE INSULIN SIGNALING CASCADE

Besides tyrosine phosphorylation (figure 2), both insulin receptor and IRS proteins have the potential to be phosphorylated at serine or threonine residues, which blocks or impairs the insulin signaling pathway⁴²⁻⁴⁴. Such inhibitory effect of serine/threonine phosphorylation is achieved by reducing the number of phosphorylated tyrosine residues^{2,43,45}, by dissociating IRS proteins from their receptor, hindering tyrosine residues phosphorylation⁴⁶, by releasing IRS from the intracellular complexes that maintain them in close proximity to the receptor⁴⁷, by promoting IRS degradation⁴⁸, or by inducing IRS interaction with other proteins rather than with the tyrosine kinase catalytic site of PI3K^{2,49}.

These inhibitory (serine/threonine) phosphorylations constitute a physiological feedback mechanism in insulin signaling⁵⁰ and allow the establishment of cross-talk mechanisms with different pathophysiological pathways that promote insulin resistance^{2,43,50}. Indeed, most of the stress and/or inflammation pathways studied so far stimulate serine/threonine phosphorylation of either IRS or insulin receptor (or both) as a way to induce insulin resistance⁵¹.

Several kinases are known to be involved in the process of serine/threonine phosphorylation-dependent regulation, namely PI3K, Akt/PKB, glycogen synthase kinase-3 (GSK3) and mammalian target of rapamycin (mTOR)², as well as PKC⁴³ and the inhibitor of nuclear factor κ (I κ B) kinase²; these last two (PKC and I κ B) have been suggested to be involved in the obesi-

ty-induced insulin resistance^{2,52,53}.

Insulin action is also attenuated by protein tyrosine phosphatases (PTPases) that promote tyrosine dephosphorylation of the insulin receptor and its substrates^{2,54}, a mechanism that seems to be augmented in many insulin resistant conditions^{2,55}, particularly in those associated with inflammation⁵⁶. Indeed, in studies using transgenic knockout of PTP1B models was observed an increase in the number of phosphorylated tyrosine residues, in both the receptor and IRS proteins, as well as an amelioration of insulin sensitivity in muscle² and liver^{57,58}, improving or avoiding the diabetic condition⁵⁹.

CONCLUSION

Insulin plays a central role in carbohydrate metabolism. Although insulin presents different effects in different target-organs, one can consider that its major role in extrahepatic tissues, such as skeletal muscle and adipose tissue, is to promote glucose uptake. The knowledge of the molecular aspects of insulin action is important to understand the mechanism underlying pathophysiology and pharmacology of insulin resistance. In the present mini-article, we provided a brief review of the main signaling pathways that ensure insulin-stimulated glucose uptake.

The insulin receptor is an obvious target molecule to pharmacologically potentiate insulin action. However, other molecules can be key players for this purpose. Akt/PKB is also a pivotal molecule for insulin signaling pathways. However, in those tissues that are dependent on insulin to acquire glucose, GLUT4 is the main glucose transporter available. Indeed, most insulin signaling pathways will ultimately lead to GLUT4 expression and/or translocation. Furthermore, even insulin-independent pathways promote glucose uptake via GLUT4 translocation. Therefore, GLUT4 can be considered as an essential key player and

target molecule for the study and/or modulation of different insulin signaling pathways involved in glucose uptake, since GLUT4 compliance should always be ensured in order to allow insulin-dependent glucose uptake.

The molecular aspects summarized herein constitute the basis for a second review, in which insulin action will be approached from a whole-body physiological perspective, more directed to the clinic.

ACKNOWLEDGEMENTS

A word of acknowledgement to Professors M. Paula Macedo and Helena Cardoso, for the help revising the present manuscript and for recognizing its scientific merit.

REFERENCES

1. Saltiel AR, Pessin JE (2002) Insulin signaling pathways in time and space. *Trends Cell Biol* **12**,65-71.
2. Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**,799-806.
3. White MF, Kahn CR (1994) The insulin signalling system. *J Biol Chem* **269**,1-4.
4. Edge ASB, Kahn RC, Spiro RG (1990) Insulin Receptor Carbohydrate Units Contain Poly-N-Acetylactosamine Chains. *Endocrinology* **127**,1887-1895.
5. Bjornholm M, Zierath JR (2005) Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes. *Biochem Soc Trans*. **33**,354-357.
6. White MF (1997) The insulin signalling system and the IRS proteins. *Diabetologia* **40 Suppl 2**,S2-17.
7. Ottensmeyer FP, Beniac DR, Luo RZ, et al. (2000) Mechanism of transmembrane signalling: insulin binding and the insulin receptor. *Biochemistry* **39**,12103-12.
8. Yip RG, Goodman HM (1999) Growth hormone and dexamethasone stimulate lipolysis and activate adenylyl cyclase in rat adipocytes by selectively shifting Gi alpha2 to lower density membrane fractions. *Endocrinology* **140**,1219-27.
9. Lawrence MC, McKern NM, Ward CW (2007) Insulin receptor structure and its implications for the IGF-1 receptor. *Current Opinion in Structural Biology Catalysis and regulation / Proteins* **17**,699-705.
10. Mosthaf L, Grako K, Dull TJ, et al. (1990) Functionally distinct insulin receptors generated by tissue-specific alternative splicing. *Embo J* **9**,2409-13.
11. Savkur RS, Philips AV, Cooper TA (2001) Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet* **29**,40-7.
12. De Meyts P (1994) The structural basis of insulin and insulin-like growth factor-I receptor binding and negative co-operativity, and its relevance to mitogenic versus metabolic signalling. *Diabetologia* **37 Suppl 2**,S135-48.
13. White TW, Srinivas M, Ripps H, et al. (2002) Virtual cloning, functional expression, and gating analysis of human connexin31.9. *Am J Physiol Cell Physiol* **283**,C960-70.
14. Whitehead JP, Clark SF, Urso B, et al. (2000) Signalling through the insulin receptor. *Curr Opin Cell Biol* **12**,222-8.
15. Carpentier JL, Paccaud JP, Backer J, et al. (1993) Two steps of insulin receptor internalization depend on different domains of the beta-subunit. *J Cell Biol* **122**,1243-52.
16. Shoelson SE, White MF, Kahn CR (1988) Tryptic activation of the insulin receptor. Proteolytic truncation of the alpha-subunit releases the beta-subunit from inhibitory control. *J Biol Chem* **263**,4852-60.
17. Villalba M, Wenthe SR, Russell DS, et al. (1989) Another version of the human insulin receptor kinase domain: expression, purification, and characterization. *Proc Natl Acad Sci U S A* **86**,7848-52.
18. Zhang B, Salituro G, Szalkowski D, et al. (1999) Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. *Science* **284**,974-7.
19. Chang L, Chiang SH, Saltiel AR (2004) Insulin signaling and the regulation of glucose transport. *Mol Med* **10**,65-71.
20. Liu YF, Herschkovitz A, Boura-Halfon S, et al. (2004) Serine phosphorylation proximal to its

- phosphotyrosine binding domain inhibits insulin receptor substrate 1 function and promotes insulin resistance. *Mol Cell Biol* **24**,9668-81.
21. Kane S, Sano H, Liu SC, *et al.* (2002) A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. *J Biol Chem* **277**,22115-8.
 22. Brozinick JT, Jr., Berkemeier BA, Elmendorf JS (2007) "Actin" on GLUT4: membrane & cytoskeletal components of insulin action. *Curr Diabetes Rev* **3**,111-22.
 23. Jordens I, Marsman M, Kuijl C, *et al.* (2005) Rab proteins, connecting transport and vesicle fusion. *Traffic* **6**,1070-7.
 24. Sano H, Kane S, Sano E, *et al.* (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. *J Biol Chem* **278**,14599-602.
 25. Ribon V, Saltiel AR (1997) Insulin stimulates tyrosine phosphorylation of the proto-oncogene product of c-Cbl in 3T3-L1 adipocytes. *Biochem J* **324 (Pt 3)**,839-45.
 26. Saito M, Lessard SJ, Rivas DA, *et al.* (2008) Activation of atypical protein kinase C[ζ] toward TC10 is regulated by high-fat diet and aerobic exercise in skeletal muscle. *Metabolism* **57**,1173-1180.
 27. Chiang SH, Baumann CA, Kanzaki M, *et al.* (2001) Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* **410**,944-8.
 28. Watson RT, Shigematsu S, Chiang SH, *et al.* (2001) Lipid raft microdomain compartmentalization of TC10 is required for insulin signaling and GLUT4 translocation. *J Cell Biol* **154**,829-40.
 29. Kanzaki M, Watson RT, Hou JC, *et al.* (2002) Small GTP-binding protein TC10 differentially regulates two distinct populations of filamentous actin in 3T3L1 adipocytes. *Mol Biol Cell* **13**,2334-46.
 30. Jiang ZY, Chawla A, Bose A, *et al.* (2002) A phosphatidylinositol 3-kinase-independent insulin signaling pathway to N-WASP/Arp2/3/F-actin required for GLUT4 glucose transporter recycling. *J Biol Chem* **277**,509-15.
 31. Maffucci T, Brancaccio A, Piccolo E, *et al.* (2003) Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation. *Embo J* **22**,4178-89.
 32. Kanzaki M, Mora S, Hwang JB, *et al.* (2004) Atypical protein kinase C (PKC[ζ]/[λ]) is a convergent downstream target of the insulin-stimulated phosphatidylinositol 3-kinase and TC10 signaling pathways. *J Cell Biol* **164**,279-290.
 33. Eguez L, Lee A, Chavez JA, *et al.* (2005) Full intracellular retention of GLUT4 requires AS160 Rab GTPase activating protein. *Cell Metab* **2**,263-72.
 34. Larance M, Ramm G, Stockli J, *et al.* (2005) Characterization of the role of the Rab GTPase-activating protein AS160 in insulin-regulated GLUT4 trafficking. *J Biol Chem* **280**,37803-13.
 35. Miinea CP, Sano H, Kane S, *et al.* (2005) AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. *Biochem J* **391**,87-93.
 36. Elmendorf JS, Pessin JE (1999) Insulin signaling regulating the trafficking and plasma membrane fusion of GLUT4-containing intracellular vesicles. *Exp Cell Res* **253**,55-62.
 37. Zeigerer A, McBrayer MK, McGraw TE (2004) Insulin stimulation of GLUT4 exocytosis, but not its inhibition of endocytosis, is dependent on RabGAP AS160. *Mol Biol Cell* **15**,4406-15.
 38. Inoue M, Chang L, Hwang J, *et al.* (2003) The exocyst complex is required for targeting of Glut4 to the plasma membrane by insulin. *Nature* **422**,629-33.
 39. Rothman JE (1994) Mechanisms of intracellular protein transport. *Nature* **372**,55-63.
 40. Sudhof TC (1995) The synaptic vesicle cycle: a cascade of protein-protein interactions. *Nature* **375**,645-53.
 41. Kawanishi M, Tamori Y, Okazawa H, *et al.* (2000) Role of SNAP23 in insulin-induced translocation of GLUT4 in 3T3-L1 adipocytes. Mediation of complex formation between syntaxin4 and VAMP2. *J Biol Chem* **275**,8240-7.
 42. Tanti JF, Gremeaux T, Van Obberghen E, *et al.* (1994) Insulin receptor substrate 1 is phosphorylated by the serine kinase activity of phosphatidylinositol 3-kinase. *Biochem J* **304 (Pt 1)**,17-21.
 43. Waraich RS, Weigert C, Kalbacher H, *et al.* (2008) Phosphorylation of Ser357 of rat insulin receptor substrate-1 mediates adverse effects of protein kinase C-delta on insulin action in skeletal muscle cells. *J Biol Chem* **283**,11226-33.
 44. Boura-Halfon S, Zick Y (2009) Phosphorylation

- of IRS proteins, insulin action, and insulin resistance. *Am J Physiol Endocrinol Metab* **296**,E581-591.
45. Tanti JF, Gremeaux T, van Obberghen E, *et al.* (1994) Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J Biol Chem* **269**,6051-7.
 46. Aguirre V, Werner ED, Giraud J, *et al.* (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* **277**,1531-7.
 47. Tzatsos A, Kandror KV (2006) Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. *Mol Cell Biol* **26**,63-76.
 48. Greene MW, Sakaue H, Wang L, *et al.* (2003) Modulation of insulin-stimulated degradation of human insulin receptor substrate-1 by Serine 312 phosphorylation. *J Biol Chem* **278**, 8199-211.
 49. Craparo A, Freund R, Gustafson TA (1997) 14-3-3 (epsilon) interacts with the insulin-like growth factor I receptor and insulin receptor substrate 1 in a phosphoserine-dependent manner. *J Biol Chem* **272**,11663-9.
 50. Boura-Halfon S, Zick Y (2009) Serine kinases of insulin receptor substrate proteins. *Vitam Horm* **80**,313-49.
 51. Tanti JF, Jager J (2009) Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. *Curr Opin Pharmacol.*
 52. Yuan M, Konstantopoulos N, Lee J, *et al.* (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* **293**,1673-7.
 53. Kim JK, Kim YJ, Fillmore JJ, *et al.* (2001) Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* **108**,437-46.
 54. Venable CL, Frevert EU, Kim YB, *et al.* (2000) Overexpression of protein-tyrosine phosphatase-1B in adipocytes inhibits insulin-stimulated phosphoinositide 3-kinase activity without altering glucose transport or Akt/Protein kinase B activation. *J Biol Chem* **275**,18318-26.
 55. Delibegovic M, Zimmer D, Kauffman C, *et al.* (2009) Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress. *Diabetes* **58**,590-9.
 56. Zabolotny JM, Kim YB, Welsh LA, *et al.* (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. *J Biol Chem* **283**,14230-41.
 57. Haj FG, Zabolotny JM, Kim YB, *et al.* (2005) Liver-specific protein-tyrosine phosphatase 1B (PTP1B) re-expression alters glucose homeostasis of PTP1B-/-mice. *J Biol Chem* **280**,15038-46.
 58. Delibegovic M, Bence KK, Mody N, *et al.* (2007) Improved Glucose Homeostasis in Mice with Muscle-Specific Deletion of Protein-Tyrosine Phosphatase 1B, 10.1128/MCB.00959-07. *Mol. Cell. Biol.* **27**, 7727-7734.
 59. Xue B, Kim YB, Lee A, *et al.* (2007) Protein-tyrosine phosphatase 1B deficiency reduces insulin resistance and the diabetic phenotype in mice with polygenic insulin resistance. *J Biol Chem* **282**,23829-40.
 60. Afonso RA (2009) Sensibilidade à Insulina Pós-prandial: Mecanismos Fisiológicos de Ativação e Fisiopatologia na Obesidade. *Faculdade de Ciências Médicas, Universidade Nova de Lisboa Tese de Doutoramento.*