

Positive and negative regulation of glucose uptake by hyperosmotic stress

P Gual, Y Le Marchand-Brustel, JF Tanti

SUMMARY

This review will provide insight on the current understanding of the intracellular signaling mechanisms by which hyperosmolarity mimics insulin responses such as Glut 4 translocation and glucose transport but also antagonizes insulin effects. Glucose uptake induced by insulin is largely dependent on the PI 3-kinase/PKB pathway. In both adipocyte and muscle cells, hyperosmolarity promotes glucose uptake by multiple mechanisms which do not require PI 3-kinase/PKB pathway but are dependent on the cell type. In muscle, osmotic stress induces glucose uptake by stimulation of AMP-Kinase and/or inhibition of Glut 4 endocytosis. In adipocytes, activation of Gab1-dependent signaling pathway plays an important role in osmotic stress-mediated glucose uptake. Apart of its insulin-like effects, hyperosmolarity can lead to cellular insulin resistance mediated by both prevention of PKB activation and inhibition of the Insulin Receptor Substrate-1 (IRS1) function. Serine phosphorylation and degradation of IRS1 negatively regulate its functions. Understanding how osmotic stress induces glucose transport or mediates insulin resistance may provide novel targets for strategies to enhance glucose transport or to prevent insulin resistance.

Key-words: Stress · Signaling · Glut 4 · Glucose uptake · Insulin resistance.

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RÉSUMÉ

Régulation positive et négative du transport du glucose par le stress hyperosmolaire

Le stress hyperosmolaire a un double effet : il mime l'effet de l'insuline sur la translocation des transporteurs de glucose Glut 4 et sur le transport du glucose, mais il inhibe aussi les effets de l'insuline sur ces mêmes paramètres. Cette revue décrit les mécanismes intracellulaires par lesquels ces effets s'exercent. La captation de glucose induite par l'insuline est largement dépendante de la stimulation de la voie PI 3-kinase/PKB dans le muscle et l'adipocyte. Si l'hyperosmolarité induit également la captation de glucose, cet effet s'exerce par de multiples mécanismes qui sont indépendants de cette voie PI 3-kinase/PKB et qui diffèrent dans le muscle squelettique et dans l'adipocyte. Dans les lignées musculaires, l'augmentation du transport de glucose en réponse à un stress hyperosmolaire implique l'activation de l'AMP-Kinase et également une inhibition de l'endocytose des transporteurs de glucose Glut 4. Dans les lignées adipocytaires, c'est l'activation d'une voie dépendante de Gab-1 qui explique l'effet de l'hyperosmolarité. Indépendamment de ses effets insulino-mimétiques, l'hyperosmolarité provoque également une insulino-résistance au niveau cellulaire qui s'explique d'une part par une désactivation de la PKB et d'autre part par une inhibition de la fonction d'IRS1. Cette rétro-régulation du signal insulinique est due à court terme à une phosphorylation d'IRS1 sur des résidus sérine et à long terme à une dégradation d'IRS1, deux processus que l'on retrouve dans l'insulino-résistance. Une meilleure connaissance des voies stimulées par le stress hyperosmolaire pour augmenter le transport de glucose ainsi que des mécanismes mis en jeu dans l'apparition de l'insulino-résistance peut fournir de nouvelles pistes et cibles thérapeutiques du diabète et de l'insulino-résistance.

Mots-clés : Stress · Signalisation · Glut 4 · Transport du glucose · Insulino-résistance.

INSERM U 568 and IFR 50, Faculté de Médecine, Avenue de Valombrose, 06107 Nice Cedex 02.

Address correspondence and reprint requests to:
P Gual, INSERM U 568, Faculté de Médecine, Avenue de Valombrose,
06107 Nice Cedex 02.
gual@unice.fr

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Inulin regulates blood glucose levels through multiple regulatory mechanisms such as suppression of endogenous glucose production by the liver and stimulation of glucose uptake into muscle and adipocytes. Glucose transport in muscle and adipose tissues is due to the translocation of the glucose transporter Glut 4 from an intracellular pool to the plasma membrane [1]. These biological responses require tyrosine phosphorylation of IRS1, which leads to, in turn, binding and activation of PI 3-kinase. Downstream effectors of PI 3-kinase such as Protein Kinase B (PKB) or atypical PKC are involved in Glut 4 translocation. Furthermore, it has been recently shown that insulin-induced Glut 4 translocation also requires the activation of a second pathway which is completely independent of PI 3-kinase activity. In adipocytes, the Cbl proto-oncogene, associated with CAP (Cbl-associated protein) and APS (adapter protein containing PH and SH2 domain) is phosphorylated in response to insulin and regulates glucose uptake. Once phosphorylated, Cbl recruits the adapter protein Crk-II in a complex with C3G, a GDP to GTP exchange factor for TC10, a Rho-family GTPase, allowing for its activation which regulates the traffic of Glut 4-containing vesicles. GTP-bound TC10 could participate in Glut 4 translocation through a modification of cortical actin or a stimulation of actin polymerization at the level of Glut 4 compartments [2-4] (*Fig 1*).

Various other stimuli can promote Glut 4 translocation through insulin— and PI 3-kinase— independent mechanisms. In skeletal muscle, contraction and hypoxia can stimulate glucose uptake [5-7]. Insulin and contraction have fully or partially additive effects on glucose transport indicating that both stimuli induce glucose uptake *via* distinct mechanisms [5, 8]. In L6 myocytes and 3T3-L1 adipocytes, inhibition of oxidative phosphorylation by 2,4-dinitrophenol or rotenone stimulates glucose transport [9-11]. Metformin, which is widely used for the therapy of type 2 diabetes mellitus, and adiponectin, a hormone secreted by the adipocytes, stimulate the uptake of glucose in muscle [12-15]. Finally, hyperosmolarity can also mimic, at least in part, insulin ef-

fect on Glut 4 translocation and glucose uptake in muscle and 3T3-L1 adipocytes [16-20]. Hyperosmotic stress-induced glucose uptake is largely independent of IRS1 phosphorylation and activation of the PI 3-kinase/PKB pathway [16, 17, 20-24]. Interestingly, hyperosmolarity induces this response *via* distinct mechanisms depending on the cell type. Furthermore, like several other insulinomimetic agents, hyperosmolarity does not only partially activate several insulin-specific biological responses but also induces a state of insulin resistance [22, 25]. We first review, in the present article, the molecular mechanisms of Glut 4-translocation and glucose uptake induced by hyperosmotic stress. We then examine by which mechanism(s) hyperosmotic stress antagonizes insulin responses. It should be noted that hyperosmotic stress (usually induced by high extracellular concentrations of sorbitol) is not a physiological stimulus but is used as a tool that could lead to discovery of novel molecular mechanisms of glucose transport and also to better understand the molecular mechanisms of cellular insulin resistance.

Osmotic stress stimulates glucose transport

In muscle and adipose cells, hyperosmolarity triggers the cell surface accumulation of Glut 4 leading to an increase in glucose transport. The mechanisms controlling this osmotic stress-mediated response have not yet been fully elucidated. Different molecular mechanisms have been reported and seem to be different in adipose tissues and skeletal muscle.

Effect of hyperosmotic stress in muscle cells

Two distinct intracellular pools of Glut 4 have been characterized in muscle. One is recruited by insulin and the other one by exercise. As previously described for exercise-induced glucose uptake, hyperosmolarity does not recruit Glut 4 from the insulin-sensitive pool since the time-courses of insulin— and hyperosmotic stress-induced Glut 4 translocation are different. Moreover, the v-SNARE VAMP2, a protein which is associated to the insulin sensitive Glut 4 pool and required for the fusion of the vesicles with the membrane, is not involved in the sorbitol-induced Glut 4-translocation [26]. Exercise and the adenoside analogue, AICAR, increase the activity of the AMP-activated protein kinase (AMPK) that correlates with glucose uptake in muscle cells [8, 13, 27]. Treatment of muscle cells with sorbitol also promotes the activation of AMPK [19, 27] and over-expression of a dominant-negative form of AMPK blocks the stimulation of Glut 4 translocation by hyperosmotic stress [28]. Altogether, these observations are in favor of an important role of AMPK activation in hyperosmolarity-induced glucose uptake. Following AMPK activation, different downstream pathways have been proposed. Fryer *et al.* have reported that AMPK phosphorylates and activates

Abbreviations

PI 3-kinase :	phosphoinositide 3-kinase
Gab1 :	Grb2-associated binder-1
Grb2 :	growth factor bound Protein-2
IRS :	insulin receptor substrate
ERK :	extracellular signal-regulated kinase
APS :	adapter protein containing PH and SH2 domain
CAP :	Cbl-associated protein
mTOR :	mammalian target of rapamycin
aPKC :	atypical protein kinase C
PTB :	phosphotyrosine binding
PKB :	protein kinase B
IKK :	inhibitor Kappa B kinase
PLD :	phospholipase D
PLC :	phospholipase C
AMPK :	AMP-activated protein kinase
NOS :	nitric oxide synthase
AICAR :	aminoimidazole-4-carboxamid I β riboside

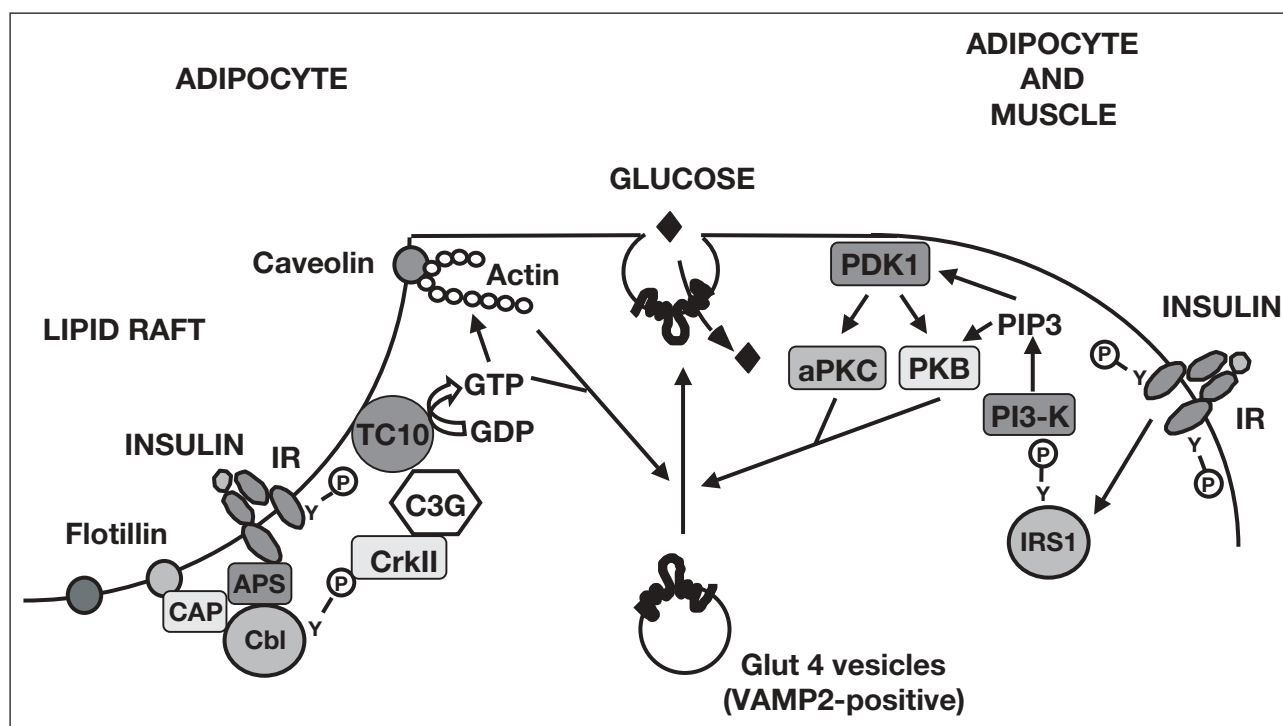


Figure 1
 Scheme of the signaling pathways used by insulin to stimulate Glut 4 translocation. Insulin binds to the insulin receptor (IR), activating its tyrosine kinase activity. The activated IR phosphorylates IRS1 on tyrosine residues allowing for the recruitment of PI3-K to the plasma membrane. PI3-kinase generates PI(3,4,5)P3 from PI(4,5)P2, thereby recruiting the 3'phosphoinositide-dependent kinase-1 (PDK-1). PDK-1 phosphorylates and activates both atypical PKC and PKB, which are required for Glut 4 translocation to the cell membrane from VAMP2-positive vesicles. In caveolae/lipid rafts of the plasma membrane, flotillin binds Cbl associated protein (CAP). CAP targets Cbl to the lipid raft domain. Cbl, also associated with APS (adapter protein containing PH and SH2 domain) is phosphorylated in response to insulin. Once phosphorylated, Cbl recruits the adapter protein Crk-II in a complex with C3G, a GDP to GTP exchange factor for TC10, allowing for its activation. Activated, GTP-bound TC10 could participate in Glut 4 translocation through a modification of cortical actin or a stimulation of actin polymerization at the level of Glut 4 compartments.

nitric oxide synthase (NOS) and that NOS inhibitors prevent sorbitol-induced glucose transport. Moreover, guanylate cyclase activity, which is increased following NO production, plays an important role in sorbitol-mediated glucose uptake [19] (Fig 2). In contrast, the group of Farese has reported that activated AMPK promotes the activation of the non-receptor proline-rich tyrosine kinase-2, PYK2, leading to the activation of the GRB2/SOS/RAS/RAF/MEK1/ERK pathway [29]. ERKs activate phospholipase D (PLD) which generates phosphatidic acid (PA). Accumulation of PA stimulates atypical PKCs, which are apparently required for the increase in Glut 4 translocation and glucose transport in response to osmotic stress [30, 31] (Fig 2).

Glut 4 accumulation at the cell surface in response to sorbitol in muscle cells seems also to be due to the inhibition of Glut 4 endocytosis, a tyrosine kinase independent process (Fig 2). This could be due to the alteration of the formation of clathrin-coated pits that are required for Glut 4 endocytosis [26].

Effect of hyperosmotic stress in adipose cells

While activation of AMPK is essential for sorbitol-induced glucose uptake in skeletal muscle, activation of AMPK by AICAR is without effect on glucose uptake in 3T3 L1-adipocytes [32]. In contrast, AMPK activation antagonizes insulin-stimulated glucose transport [33]. Furthermore, inhibition of NOS by L-NMMA or guanylate cyclase by LY83583 did not prevent hyperosmolarity-stimulated glucose uptake (personal communication). In 3T3-L1 adipocytes, glucose transport activity induced by osmotic stress is fully dependent on a tyrosine kinase activity and requires the t-SNARE protein syntaxin-4 for docking/fusion of Glut 4-containing vesicles [16, 18]. Among the proteins phosphorylated on tyrosine following sorbitol treatment, Gab-1 (Grb2-associated binder-1) seems to play a key role. Indeed, microinjection of anti-Gab-1 antibodies strongly inhibits osmotic stress-induced Glut 4 translocation without altering the insulin effect [18]. Gab-1 is an adapter

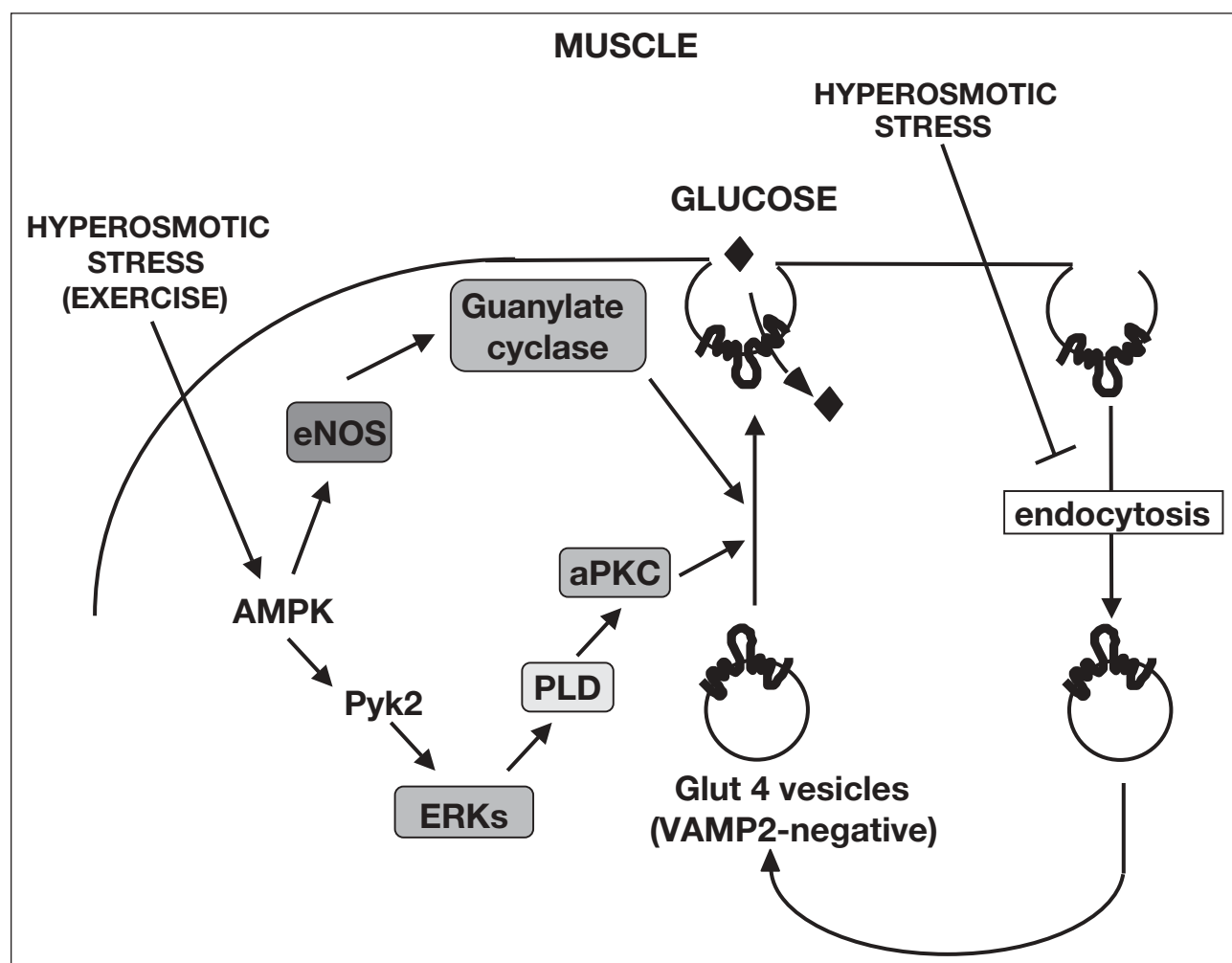


Figure 2

Signaling pathways activated by hyperosmotic stress to induce Glut 4 translocation in muscle cells. Osmotic stress increases the activity of AMPK, which, in turn, leads to the stimulation of two distinct pathways. AMPK phosphorylates and activates the eNOS leading to the production of NO and activation of guanylate cyclase. AMPK could also activate the PYK2 tyrosine kinase activity leading to the activation of ERK through the Grb2/SOS/Ras/Rak/MEK1/ERK pathway. ERK activate PLD, which generates phosphatidic acid (PA). Accumulation of PA stimulates aPKC, which plays a role in hyperosmotic stress-induced glucose uptake. Both pathways promote the translocation of Glut 4 to the cell membrane from VAMP2-negative vesicles. In addition, Glut 4 accumulation at the cell surface in response to osmotic stress also results from the inhibition of its endocytosis, a tyrosine kinase independent process. This could be due to the alteration in formation of clathrin-coated pits that are required for Glut 4 endocytosis.

protein, which possesses an N-terminal pleckstrin homology (PH) domain and 16 potential tyrosine phosphorylation sites [34, 35]. In response to osmotic stress, Gab-1 acts as a docking protein for PLC γ 1, the p85 subunit of PI3 kinase and Crk-II. Both Gab1-dependent PI 3-kinase and PLC- γ activities appear to play a role in osmotic stress-induced membrane ruffling [18] and gene expression [36], respectively, but are not required for glucose transport [16, 17, 20-24]. In contrast, the formation of Gab1-Crk-II complex correlates with Glut 4 translocation and glucose uptake [20]. Upon sorbitol stimulation, the phosphorylated Gab1 recruits Crk-II *via* its SH2 domain. The Crk-II SH3 domains are constitutively associated with C3G, a GDP to GTP exchange factor for several small GTP-binding proteins including TC10. In fa-

vor of this pathway, we have shown that osmotic stress-mediated Glut 4 translocation and glucose uptake are inhibited by overexpression of the dominant-interfering TC10/T31N mutant or by inactivation of TC10 by *clostridium difficile* toxin B [20]. The full activation of CrkII/TC10 pathway is mediated via Gab1 but could be also dependent on Cbl, which is phosphorylated on tyrosine residue in response to sorbitol [16]. However, signaling pathways downstream of Gab1, and specifically the Gab1/CrkII/C3G/TC10 signaling, play a crucial role in osmotic-stress induced glucose transport [18, 20] (Fig 3). Since Gab1, CrkII/C3G, TC10 are expressed in muscle [34, 37-38], it is possible that osmotic stress-stimulated glucose uptake could also be mediated by this signaling pathway.

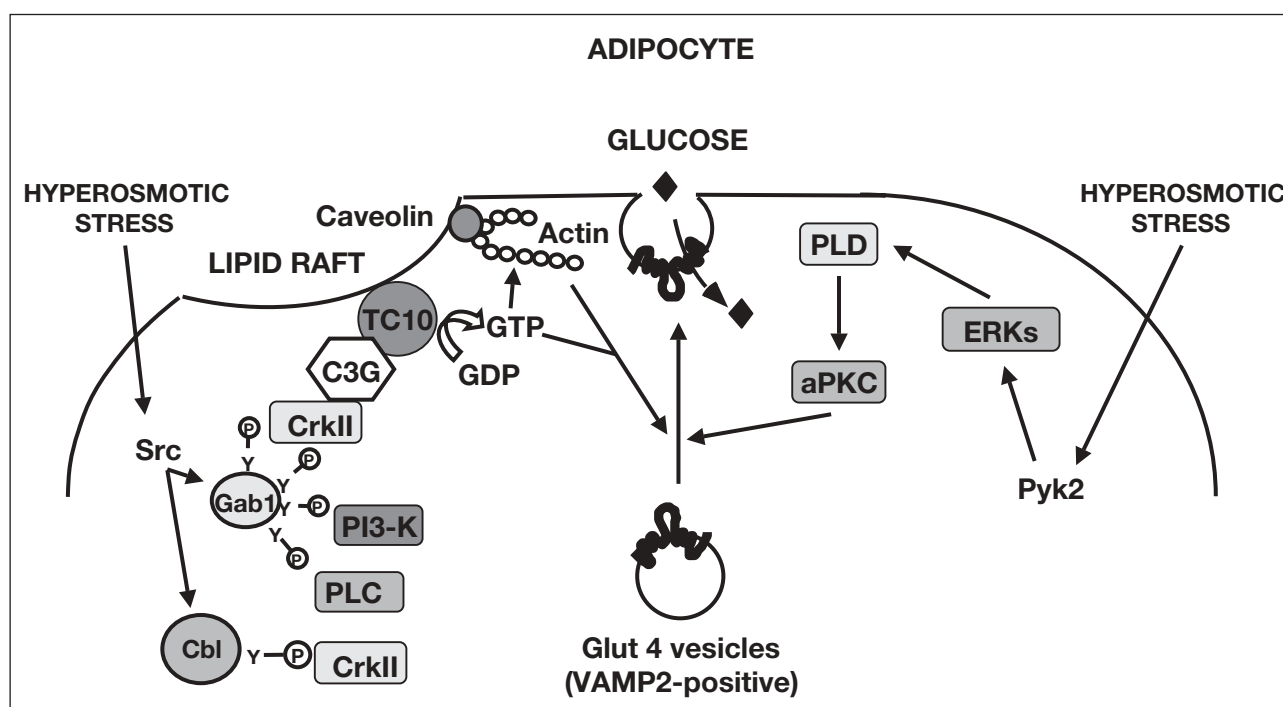


Figure 3 Signaling pathways activated by hyperosmotic stress to induce Glut 4 translocation in 3T3-L1 adipocytes and adipose cells. Osmotic stress induces glucose uptake by activation of Gab1 dependent signaling pathways. Sorbitol promotes the activation of cytosolic Src kinase, which phosphorylates Gab1 on tyrosine residue. Phosphorylated Gab1 recruits the CrkII/C3G complex. C3G, a guanine nucleotide exchange factor, exchanges GDP for GTP on TC10. Activated GTP bound TC10 could then modify the cortical actin structure or stimulate the actin polymerization on Glut 4 compartments. Osmotic stress recruits vesicles containing both Glut 4 and VAMP2 in adipocytes. Glut 4 translocation stimulated by hyperosmotic stress could also depend on the PYK2 activity, which leads to the activation of ERK, PLD and finally atypical PKC.

As described above for muscle cells, it has been reported similarly that hyperosmotic stress activates the PYK2/ERK/PLD/aPKC pathway in 3T3 L1 adipocytes and adipose cells, leading to Glut 4 translocation and glucose uptake [30] (Fig 3). However, several reports are not in favor of a role of ERK activation in osmotic stress-induced glucose uptake in 3T3 L1-adipocytes. Indeed, inhibition of ERK activity by cell treatment with a MEK1 inhibitor PD98059 or expression of a dominant-interfering mutant Δ SOS, did not change the effect of osmotic stress on glucose transport [17, 20].

In adipose cells from type 2 diabetic subjects, insulin-signaling pathways are severely impaired, including IRS1 phosphorylation, PKB activity, and glucose transport [39]. In these cells, activation of Gab1-signaling pathway is still responsive to growth factor [40]. Since human insulin resistance correlates with inhibition of IRS1 function, the activation by pharmacological agents of pathways dependent on Gab1 could overcome the inhibition of IRS1 function and partially or totally rescue glucose transport.

While a unifying mechanism of hyperosmotic stress induced glucose uptake has not yet been identified, the new emerging pathways involved in this response give a novel viewpoint of potential insulin-independent mechanisms for glucose uptake.

Hyperosmotic stress leads to cellular insulin resistance

The earliest abnormality observed in insulin resistance is a decrease in the insulin-induced glucose uptake in skeletal muscle and adipose tissue and a reduced ability of the hormone to suppress hepatic glucose production [41, 42]. The inability of insulin to promote normal cellular glucose uptake could result from both a decrease in the cellular Glut 4 gene expression and the alteration in insulin signaling pathways controlling Glut 4 translocation and glucose uptake [3].

In rat epididymal adipose cells, hyperosmotic stress markedly reduces insulin-induced glucose transport [43]. Similarly, pretreatment of 3T3-L1 adipocytes with sorbitol strongly decreases the ability of insulin to stimulate glucose uptake, lipogenesis and glycogen synthesis [22]. Concomitant molecular mechanisms by which hyperosmotic stress antagonizes insulin-mediated responses have been reported, that we will review below.

Inactivation of PKB

Chen *et al.* have reported that hyperosmolarity prevents insulin-induced PKB activation. This suggests that stimulation of a calyculin A- or okadaic acid-sensitive protein phos-

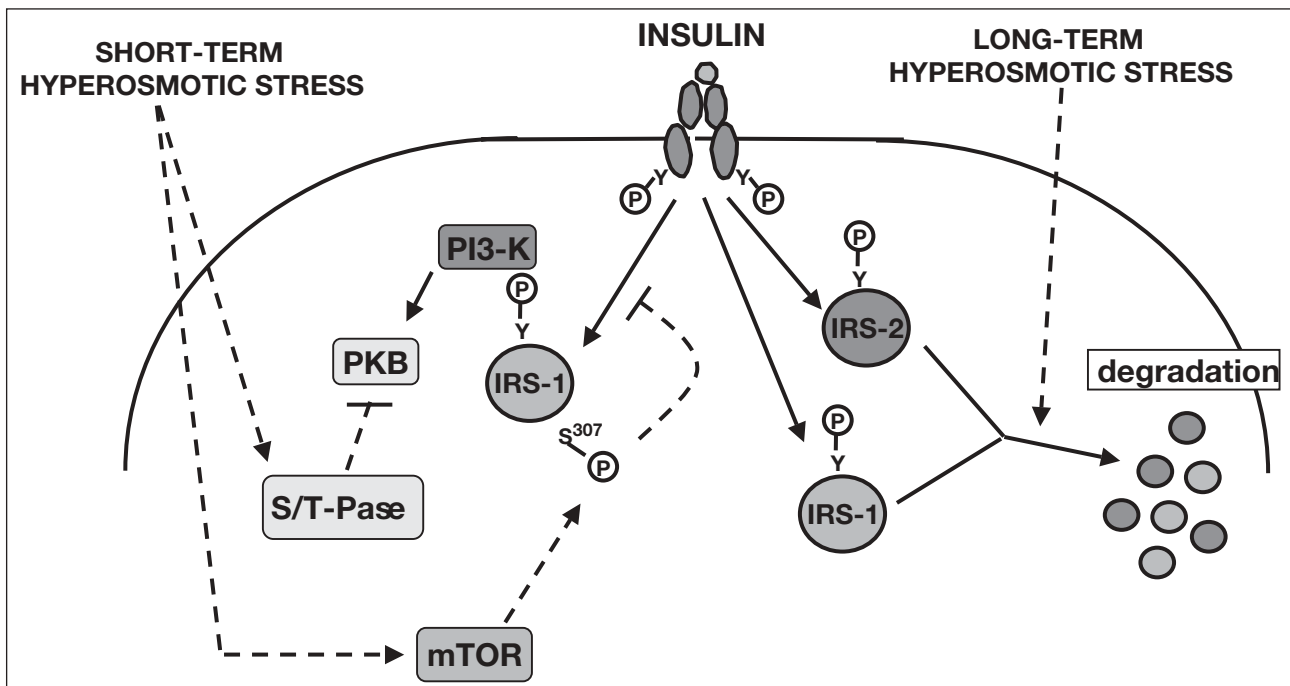


Figure 4

Mechanisms of cellular insulin resistance induced by hyperosmotic stress. Acute osmotic stress induces the phosphorylation of IRS1 on Ser³⁰⁷ by a mTOR-dependent pathway. This, in turn, leads to an impairment of IRS1 functions induced by physiological insulin concentrations. Hyperosmotic stress promotes also the activation of a PKB phosphatase that maintains PKB in an inactive state in response to insulin. On the other hand, prolonged osmotic stress alters the IRSs function by inducing their degradation, which could contribute to the downregulation of insulin action. Insulin signaling pathways are represented in full-lines while the effects of hyperosmotic stress are represented in dotted lines.

phatase leads to the deactivation of PKB [22] (Fig 4). A strong inhibition of insulin-mediated PKB activation has also been detected in adipocytes from diabetic patients and their non-diabetic relatives, which could be attributed to 70% reduction in IRS1 protein expression and PI 3-kinase activity. In contrast, the activity of PKB appears to be normal or only impaired *in vitro* in skeletal muscle from the same subjects [39]. Since the impairment of insulin signaling at the level of PKB is specific for adipose tissue from diabetic patients, identification of a unifying mechanism leading to inhibition of glucose uptake could provide attractive targets for strategies to prevent insulin resistance.

Serine phosphorylation of IRS1

In addition to dephosphorylation of PKB, another mechanism is involved in hyperosmolarity-induced insulin resistance. Indeed, in 3T3-L1 adipocytes, hyperosmotic stress inhibits both tyrosine phosphorylation of IRS1 and IRS1-associated PI 3-kinase activity in response to physiological insulin concentrations [25]. One attractive explanation is that hyperosmotic stress negatively regulates the function of IRS1 by increasing its serine phosphorylation. These post-translational modifications could be a general mechanism to regulate the functions of docking proteins (IRS1, Gab1, Gab2) [44-47].

The concept that IRS1 Ser phosphorylation impairs insulin action has emerged ten years ago when Tanti *et al.* 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 [44, 48]. This effect was linked to a decrease in IRS1 tyrosine phosphorylation and PI 3-kinase activation. We found that okadaic acid markedly increases the Ser phosphorylation of IRS1 and that hyperphosphorylated IRS1 is a poor substrate for insulin receptor [44]. Emerging data demonstrate that several factors such as PDGF, chronic insulin stimulation or TNF- Δ , which are implicated in insulin resistance, use this process to down-regulate insulin signaling [49-53]. The mechanism by which serine phosphorylation of IRS1 inhibits its function has not been fully elucidated yet [54]. However, it has been shown that phosphorylation of serine 307 could play an important role. Ser³⁰⁷ is located close to the phosphotyrosine binding (PTB) domain of IRS1, which is involved in its interaction with activated insulin receptor. Phosphorylation of Ser³⁰⁷ could then induce a conformational change, which prevents the association between IRS1 and insulin receptor leading to a decrease in IRS1 tyrosine phosphorylation [55].

In 3T3-L1 adipocytes, hyperosmotic stress inhibits the IRS1 functions in response to insulin. As a consequence,

insulin-induced membrane ruffling, which is dependent on PI 3-kinase activation, is markedly reduced. Interestingly, these inhibitory effects are associated with an increase in the phosphorylation of IRS1 on Ser³⁰⁷ residue. This effect is completely dependent on mammalian target of rapamycin (mTOR). Indeed, rapamycin, the mTOR inhibitor, prevents the osmotic stress-induced phosphorylation of IRS1 on Ser³⁰⁷ and reverses the inhibitory effect of hyperosmotic stress on insulin-induced IRS1 tyrosine phosphorylation and PI 3-kinase activation [25] (*Fig 4*). However, it is interesting to note that a supraphysiological concentration of insulin (100 nM), which leads to the activation of a high amount of insulin receptors, overcomes the inhibitory effect of osmotic stress on IRS1 function [22, 25]. This indicates that a delicate balance between positive IRS1 tyrosine phosphorylation *versus* negative IRS1 serine phosphorylation could regulate the IRS1 function.

The importance of the Ser³⁰⁷ in the negative regulation of IRS1 function seems to be a general mechanism. Several stimuli such as fatty acids [56], chronic administration of insulin or IGF-1 [52, 57-58] and TNF- α [57, 59] induce the phosphorylation of Ser³⁰⁷ leading to the reduction in both IRS1 tyrosine phosphorylation and IRS1-associated PI 3-kinase activity. However, the phosphorylation of Ser³⁰⁷ could be catalyzed by multiple kinases. In response to TNF- α , both the c-Jun amino terminal kinase (JNK) and the inhibitor κ B kinase- β (IKK- β) are involved [60-63]. In response to insulin, we and others have found the mTOR signaling pathway as predominantly involved in insulin-induced phosphorylation of Ser³⁰⁷ in adipocytes, muscles and hepatocytes [52, 58], although JNK could also be involved [62]. In response to fatty acids, the phosphorylation of Ser³⁰⁷ was correlated with an increase in the activity of PKC- θ [56, 64], but JNK could also be involved [61].

Since IRS1 contains nearly 100 potential serine phosphorylation sites, other serine phosphorylation sites and kinases are likely involved in the inhibition of IRS1 functions. We recently determined that hyperosmotic stress also promotes the phosphorylation of Ser⁶³² (personal communication). Furthermore, several groups identified that Ser⁶¹² and Ser⁶³² are also phosphorylated in response to insulin and TNF- α . Both serine residues are located close to tyrosine residues which are major phosphorylation sites involved in the binding of PI 3-kinase and 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 IRS1 and PI 3-kinase and/or its activation [65, 66]. The phosphorylation of these sites is mediated by MAPK and/or mTOR signaling pathways in response to both insulin [52] or TNF α [63, 67]. We recently reported that the basal level of IRS1 phosphorylation on Ser⁶³² (corresponding to Ser⁶³⁶ in human IRS1 sequence) and MAPK activity are abnormally high in primary cultures of skeletal muscle cells

obtained from type 2 diabetic patients [68]. Concomitantly, insulin-induced IRS1 tyrosine phosphorylation was altered. Moreover, inhibition of MAPK normalized the level of IRS1 Ser⁶³² phosphorylation [68]. These results favor a role of MAPK in the decrease in insulin sensitivity in type 2 diabetic patients.

Enhanced degradation of IRS proteins

While serine phosphorylation is usually considered as a short term inhibitory mechanism, regulated degradation of IRS proteins might also promote long term insulin-resistance. Adipocytes from type 2 diabetic subjects, obese patients and relatives of diabetic subjects display a 70% decrease in IRS1 protein expression. In contrast, no significant decrease in IRS2 protein expression has been reported. Likewise, IRS1 protein expression appears to be unchanged in skeletal muscle or primary cultures of skeletal muscle cells from either type 2 diabetic subjects or relatives of diabetic patients. A small (30%) reduction in IRS1 protein expression was reported in morbidly obese subjects [39].

In 3T3-L1 adipocytes, prolonged osmotic stress promotes the degradation not only of IRS1 but also of IRS2 (*Fig 4*) [25]. Oxidative stress, a putative causative factor for cellular insulin resistance [69, 70], also leads to IRS1 degradation [71]. In contrast to recent data [72], we have shown that osmotic stress stimulates IRS2 degradation through a proteasome-independent process [25]. The decrease in IRS1 protein expression by oxidative stress is also insensitive to proteasome inhibitors [71]. This could indicate that both oxidative and osmotic stress induce these effects through a lysosomal process. In contrast, the prolonged treatment of adipocytes with insulin reduces the level of IRS1 through a proteasome-dependent process. Moreover, it has been proposed that the mTOR-dependent IRS1 phosphorylation on serine could allow for its degradation [51, 73-75]. In conclusion, although both insulin and stress (oxidative and osmotic) induce the serine phosphorylation of IRS1, other events activated only by insulin treatment could be required to trigger IRS1 degradation by the proteasome. Since the N-terminal region of IRS1 contains a structural element that is crucial for the specificity of ubiquitination and proteasome degradation in response to insulin [76], another region of this protein could be required for stress-induced degradation.

In conclusion, insulin resistance is a very complex phenomenon, which includes defects at the level of insulin receptor and also at the various steps of insulin action leading to glucose transport (IRS1 phosphorylation, PI 3-kinase activity, Glut 4 gene expression, IRS1 protein expression...). More studies are required to better understand the mechanism of insulin resistance in its entirety. This review provides hypothetical targets for novel strategies to enhance glucose transport or to prevent insulin resistance. Stimulation of Glut 4 translocation to cell membrane through insulin-independent mechanisms could enhance glucose uptake in

insulin-resistant patients. Likewise, inhibition of specific kinases involved in impairment of IRS1 functions could increase the insulin sensitivity.

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References

1. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, 2001, 414, 799-806.
2. Saltiel AR, Pessin JE. Insulin signaling pathways in time and space. *Trends Cell Biol*, 2002, 12, 65-71.
3. Ducluzeau PH, Fletcher LM, Vidal H, Laville M, Tavares JM. Molecular mechanisms of insulin-stimulated glucose uptake in adipocytes. *Diabetes Metab*, 2002, 28, 85-92.
4. Khan AH, Pessin JE. Insulin regulation of glucose uptake : a complex interplay of intracellular signalling pathways. *Diabetologia*, 2002, 45, 1475-83.
5. Sakamoto K, Goodyear LJ. Intracellular signaling in contracting skeletal muscle. *J Appl Physiol*, 2002, 93, 369-83.
6. Azevedo JL Jr, Carey JO, Pories WJ, Morris PG, Dohm GL. Hypoxia stimulates glucose transport in insulin-resistant human skeletal muscle. *Diabetes*, 1995, 44, 695-8.
7. Cartee GD, Douen AG, Ramlal T, Klip A, Holloszy JO. Stimulation of glucose transport in skeletal muscle by hypoxia. *J Appl Physiol*, 1991, 70, 1593-600.
8. Mu J, Brozinick JT Jr, Valladares O, Bucan M, Birnbaum MJ. A role for AMP – activated protein kinase in contraction – and hypoxia-regulated glucose transport in skeletal muscle. *Mol Cell*, 2001, 7, 1085-94.
9. Bashan N, Burdett E, Guma A, *et al*. Mechanisms of adaptation of glucose transporters to changes in the oxidative chain of muscle and fat cells. *Am J Physiol*, 1993, 264, 430-40.
10. Khayat ZA, Tsakiridis T, Ueyama A, Somwar R, Ebina Y, Klip A. Rapid stimulation of glucose transport by mitochondrial uncoupling depends in part on cytosolic Ca²⁺ and cPKC. *Am J Physiol*, 1998, 275, 1487-97.
11. Tsakiridis T, Vranic M, Klip A. Phosphatidylinositol 3-kinase and the actin network are not required for the stimulation of glucose transport caused by mitochondrial uncoupling : comparison with insulin action. *Biochem J*, 1995, 309, 1-5.
12. Hundal HS, Ramlal T, Reyes R, Leiter LA, Klip A. Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. *Endocrinology*, 1992, 131, 1165-73.
13. Zhou G, Myers R, Li Y, *et al*. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*, 2001, 108, 1167-74.
14. Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ. Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes*, 2003, 52, 1355-63.
15. Yamauchi T, Kamon J, Minokoshi Y, *et al*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med*, 2002, 8, 1288-95.
16. Chen D, Elmendorf JS, Olson AL, Li X, Earp HS, Pessin JE. Osmotic shock stimulates GLUT4 translocation in 3T3L1 adipocytes by a novel tyrosine kinase pathway. *J Biol Chem*, 1997, 272, 27401-10.
17. Sakaue H, Ogawa W, Takata M, Kuroda S, Kotani K, Matsumoto M, Sakaue M, *et al*. Phosphoinositide 3-kinase is required for insulin-induced but not for growth hormone- or hyperosmolarity-induced glucose uptake in 3T3-L1 adipocytes. *Mol Endocrinol*, 1997, 11, 1552-62.
18. Janez A, Worrall DS, Imamura T, Sharma PM, Olefsky JM. The osmotic shock-induced glucose transport pathway in 3T3-L1 adipocytes is mediated by Gab-1 and requires Gab-1-associated phosphatidylinositol 3-kinase activity for full activation. *J Biol Chem*, 2000, 275, 26870-6.
19. Fryer LG, Hajduch E, Rencurel F, *et al*. Activation of glucose transport by AMP-activated protein kinase *via* stimulation of nitric oxide synthase. *Diabetes*, 2000, 49, 1978-85.
20. Gual P, Shigematsu S, Kanzaki M, Grémeaux T, Gonzalez T, Pessin JE, Le Marchand-Brustel Y, Tanti JF. A Crk-II/TC10 signaling pathway is required for osmotic shock-stimulated glucose transport. *J Biol Chem*, 2002, 277, 43980-6.
21. Meier R, Thelen M, Hemmings BA. Inactivation and dephosphorylation of protein kinase B α (PKB α) promoted by hyperosmotic stress. *EMBO J*, 1998, 17, 7294-303.
22. Chen D, Fucini RV, Olson AL, Hemmings BA, Pessin JE. Osmotic shock inhibits insulin signaling by maintaining Akt/protein kinase B in an inactive dephosphorylated state. *Mol Cell Biol*, 1999, 19, 4684-94.
23. Kayali AG, Austin DA, Webster NJ. Stimulation of MAPK cascades by insulin and osmotic shock : lack of an involvement of p38 mitogen-activated protein kinase in glucose transport in 3T3-L1 adipocytes. *Diabetes*, 2000, 49, 1783-93.
24. Barros LF, Barnes K, Ingram J, Castro J, Porras OH, Baldwin SA. Hyperosmotic shock induces both activation and translocation of glucose transporters in mammalian cells. *Pflugers Arch*, 2001, 442, 614-21.
25. Gual P, Gonzalez T, Grémeaux T, Barrès R, Le Marchand-Brustel Y, Tanti JF. Hyperosmotic stress inhibits IRS1 function by distinct mechanisms in 3T3-L1 adipocytes. *J Biol Chem*, 2003, 278, 26550-7.
26. Li D, Randhawa VK, Patel N, Hayashi M, Klip A. Hyperosmolarity reduces GLUT4 endocytosis and increases its exocytosis from a VAMP2-independent pool in L6 muscle cells. *J Biol Chem*, 2001, 276, 22883-91.
27. Hayashi T, Hirshman MF, Fujii N, Habinowski SA, Witters LA, Goodyear LJ. Metabolic stress and altered glucose transport : activation of AMP-activated protein kinase as a unifying coupling mechanism. *Diabetes*, 2000, 49, 527-31.
28. Fryer LG, Fougelle F, Barnes K, Baldwin SA, Woods A, Carling D. Characterization of the role of the AMP-activated protein kinase in the stimulation of glucose transport in skeletal muscle cells. *Biochem J*, 2002, 363, 167-74.
29. Chen HC, Bandyopadhyay G, Sajjan MP, *et al*. Activation of the ERK pathway and atypical protein kinase C isoforms in exercise – and aminoimidazole-4-carboxamide-1-beta-D-ribose (AICAR) – stimulated glucose transport. *J Biol Chem*, 2002, 277, 23554-62.

30. Sajan MP, Bandyopadhyay G, Kanoh Y, *et al.* Sorbitol activates atypical protein kinase C and GLUT4 glucose transporter translocation/glucose transport through proline-rich tyrosine kinase-2, the extracellular signal-regulated kinase pathway and phospholipase D. *Biochem J*, 2002, 362, 665-74.
31. Farese RV. Function and dysfunction of aPKC isoforms for glucose transport in insulin-sensitive and insulin-resistant states. *Am J Physiol Endocrinol Metab*, 2002, 283, 1-11.
32. Sakoda H, Ogihara T, Anai M, *et al.* Activation of AMPK is essential for AICAR-induced glucose uptake by skeletal muscle but not adipocytes. *Am J Physiol Endocrinol Metab*, 2002, 282, 1239-44.
33. Salt IP, Connell JM, Gould GW. 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes. *Diabetes*, 2000, 49, 1649-56.
34. Holgado-Madruga M, Emlt DR, Moscatello DK, Godwin AK, Wong AJ. A GRB2-associated docking protein in EGF- and insulin-receptor signalling. *Nature*, 1996, 379, 560-4.
35. Weidner KM, DiCesare S, Sachs M, Brinkmann V, Behrens J, Birchmeier W. Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature*, 1996, 384, 173-6.
36. Kultz D, Chakravarty D. Maintenance of genomic integrity in mammalian kidney cells exposed to hyperosmotic stress. *Comp Biochem Physiol A Mol Integr Physiol*, 2001, 130, 421-8.
37. Feller SM. Crk family adaptors-signalling complex formation and biological roles. *Oncogene*, 2001, 20, 6348-71.
38. Neudauer CL, Joberty G, Tatsis N, Macara IG. Distinct cellular effects and interactions of the Rho-family GTPase TC10. *Curr Biol*, 1998, 8, 1151-60.
39. Smith U. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance-is insulin resistance initiated in the adipose tissue? *Int J Obes Relat Metab Disord*, 2002, 26, 897-904.
40. Gogg S, Smith U. Epidermal growth factor and transforming growth factor α mimic the effects of insulin in human fat cells and augment downstream signaling in insulin resistance. *J Biol Chem*, 2002, 277, 36045-51.
41. DeFronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. *Diabetes Review*, 1997, 5, 177-269.
42. White MF. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab*, 2002, 283, 413-22.
43. Komjati M, Kastner G, Waldhausl W, Bratusch-Marrain P. Detrimental effect of hyperosmolality on insulin-stimulated glucose metabolism in adipose and muscle tissue *in vitro*. *Biochem Med Metab Biol*, 1988, 39, 312-8.
44. Tanti JF, Grémeaux T, Van Obberghen E, Le Marchand-Brustel Y. Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J Biol Chem*, 1994, 269, 6051-7.
45. Gual P, Giordano S, Anguissola S, Parker PJ, Comoglio PM. Gab1 phosphorylation: a novel mechanism for negative regulation of HGF receptor signaling. *Oncogene*, 2001, 20, 156-66.
46. Yu CF, Liu Z X, Cantley LG. ERK negatively regulates the epidermal growth factor-mediated interaction of Gab1 and the phosphatidylinositol 3-kinase. *J Biol Chem*, 2002, 277, 19382-8.
47. Lynch DK, Daly RJ. PKB-mediated negative feedback tightly regulates mitogenic signalling *via* Gab2. *EMBO J*, 2002, 21, 72-82.
48. Jullien D, Tanti JF, Heydrick SJ, *et al.* Differential effects of okadaic acid on insulin-stimulated glucose and amino acid uptake and phosphatidylinositol 3-kinase activity. *J Biol Chem*, 1993, 268, 15246-51.
49. Ricort JM, Tanti JF, Van Obberghen E, Le Marchand-Brustel Y. Cross-talk between the Platelet-derived Growth Factor and the insulin signaling pathways in 3T3-L1 adipocytes. *J Biol Chem*, 1997, 272, 19814-8.
50. Staubs PA, Nelson JG, Reichart DR, Olefsky JM. Platelet-derived growth factor inhibits insulin stimulation of insulin receptor substrate-1-associated phosphatidylinositol 3-kinase in 3T3-L1 adipocytes without affecting glucose transport. *J Biol Chem*, 1998, 273, 25139-47.
51. Takano A, Usui I, Haruta T, *et al.* Mammalian target of rapamycin pathway regulates insulin signaling via subcellular redistribution of insulin receptor substrate 1 and integrates nutritional signals and metabolic signals of insulin. *Mol Cell Biol*, 2001, 21, 5050-62.
52. Gual P, Gremeaux T, Gonzalez T, Le Marchand-Brustel Y, Tanti JF. MAP kinases and mTOR mediate insulin-induced phosphorylation of Insulin Receptor Substrate-1 on serine residues 307, 612 and 632. *Diabetologia*, 2003, DOI 10.1007.
53. Hotamisligil GS. The role of TNF α and TNF receptors in obesity and insulin resistance. *J Intern Med*, 1999, 245, 621-25.
54. Zick Y. Insulin resistance: a phosphorylation-based uncoupling of insulin signaling. *Trends Cell Biol*, 2001, 11, 437-41.
55. 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*, 2002, 277, 1531-7.
56. Yu C, Chen Y, Cline GW, *et al.* Mechanism by which fatty acids inhibit insulin activation of IRS-1 associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem*, 2002, 277, 50230-6.
57. Rui L, Aguirre V, Kim J, *et al.* Insulin/IGF-1 and TNF- α stimulate phosphorylation of IRS-1 at inhibitory Ser³⁰⁷ via distinct pathways. *J Clin Invest*, 2001, 107, 181-9.
58. 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*, 2003, 278, 8199-211.
59. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH2-terminal Kinase Promotes Insulin Resistance during Association with Insulin Receptor Substrate-1 and Phosphorylation of Ser³⁰⁷. *J Biol Chem*, 2000, 275, 9047-54.
60. Gao Z, Hwang D, Bataille F, *et al.* Serine Phosphorylation of Insulin Receptor Substrate 1 by Inhibitor κ B Kinase Complex. *J Biol Chem*, 2002, 277, 48115-21.
61. Hirosumi J, Tuncman G, Chang L, *et al.* A central role for JNK in obesity and insulin resistance. *Nature*, 2002, 420, 333-6.
62. 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*, 2003, 278, 2896-902.
63. Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J. Aspirin inhibits serine phosphorylation of IRS-1 in TNF-treated cells through targeting multiple serine kinases. *J Biol Chem*, 2003, 278, 24944-50.
64. Griffin ME, Marcucci MJ, Cline GW, *et al.* Free fatty acid-induced insulin resistance is associated with activation of protein kinase C θ and alterations in the insulin signaling cascade. *Diabetes*, 1999, 48, 1270-4.
65. 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*, 1996, 271, 11222-7.
66. Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C phosphorylates insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in response to insulin. *J Biol Chem*, 2001, 276, 3543-9.
67. Ozes ON, Akca H, Mayo LD, *et al.* A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. *Proc Natl Acad Sci USA*, 2001, 98, 4640-5.

68. Bouzakri K, Roques M, Gual P, *et al.* 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*, 2003, 52, 1319-25.
69. Rudich A, Tirosh A, Potashnik R, Hemi R, Kanety H, Bashan N. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. *Diabetes*, 1998, 47, 1562-9.
70. Tirosh A, Rudich A, Potashnik R, Bashan N. Oxidative stress impairs insulin but not platelet-derived growth factor signalling in 3T3-L1 adipocytes. *Biochem J*, 2001, 355, 757-63.
71. Potashnik R, Bloch-Damti A, Bashan N, Rudich A. IRS1 degradation and increased serine phosphorylation cannot predict the degree of metabolic insulin resistance induced by oxidative stress. *Diabetologia*, 2003, 46, 639-48.
72. Rui L, Fisher TL, Thomas J, White MF. Regulation of insulin/insulin-like growth factor-1 signaling by proteasome-mediated degradation of insulin receptor substrate-2. *J Biol Chem*, 2001, 276, 40362-7.
73. Sun XJ, Goldberg JL, Qiao LY, Mitchell JJ. Insulin-induced insulin receptor substrate-1 degradation is mediated by the proteasome degradation pathway. *Diabetes*, 1999, 48, 1359-64.
74. Haruta T, Uno T, Kawahara J, *et al.* A rapamycin-sensitive pathway down-regulates insulin signaling via phosphorylation and proteasomal degradation of insulin receptor substrate-1. *Mol Endocrinol*, 2000, 14, 783-94.
75. Pederson TM, Kramer DL, Rondinone CM. Serine/threonine phosphorylation of IRS-1 triggers its degradation : possible regulation by tyrosine phosphorylation. *Diabetes*, 2001, 50, 24-31.
76. Zhande R, Mitchell JJ, Wu J, Sun XJ. Molecular mechanism of insulin-induced degradation of insulin receptor substrate 1. *Mol Cell Biol*, 2002, 22, 1016-26.