Molecular Mechanisms of Insulin Resistance: An Update

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Abstract

The biological actions of insulin are initiated by activating its membrane receptor, which triggers multiple signaling pathways to mediate their biological actions. Due to the importance of metabolic regulation and promoting functions of cell growth and proliferation, insulin actions are highly regulated to promote proper metabolic functioning and energy balance. If these mechanisms are altered, this can lead to a condition known as insulin resistance, which is the consequence of a deficient insulin signaling caused by mutations or post-translational modifications of the receptor or effector molecules located downstream. Insulin resistance is one of the main characteristics of pathological manifestations associated with type 2 diabetes mellitus, one of the leading causes of death in Mexico and worldwide. In recent years, it has been found that conditions such as inflammation, endoplasmic reticulum stress, and mitochondrial dysfunction promote insulin resistance. The aim of this review is to elucidate the molecular aspects of insulin resistance and the mechanisms involved in regulating its effects, with particular emphasis on the role of inflammation, endoplasmic reticulum stress, and mitochondrial dysfunction.

KEY WORDS: *Insulin. Insulin resistance. Inflammation. Endoplasmic reticulum stress. Mitochondrial dysfunction.*

Introduction

Type 2 diabetes mellitus (DM2) is the most common endocrine disorder in humans. According to the International Diabetes Federation (IDF), currently it affects more than 387 million people worldwide, and by the year 2035, potentially it will affect more than 592 million (http://www.idf.org). DM2, also known as "non-insulin dependent diabetes" or "adult diabetes", is a chronic-degenerative disease characterized by the presence of insulin resistance, a condition where cells that usually respond to insulin stop doing it, and/or due to relative deficiency of this hormone in the body. Insulin, which carries out vital functions especially in energy metabolism, also participates in the regulation of different processes at the cardiovascular level and in the central nervous system (CNS)[1,2].

Clinical and experimental trials have provided evidence that insulin resistance in metabolic tissues, such as adipose, hepatic and muscular tissues, constitutes a characteristic trait of metabolic dysfunction, mainly induced by obesity[3]. This peripheral insulin resistance causes pancreatic β cells to secrete more insulin, in a process known as compensatory hyperinsulinemia. However, together with insulin resistance, often there is β cell depletion, which results in sustained hyperglycemia and DM2[1,3]. Besides, insulin resistance importantly contributes to the development of other conditions such as dyslipidemia, hypertension and atherosclerosis. At the molecular level, insulin resistance is the consequence of this hormone's signaling alterations, owing to mutations or post-translational modifications of its receptor or downstream-located effector proteins[4].

Given that insulin resistance plays a fundamental role in DM2 pathogenesis, considerable efforts have been made to elucidate responsible factors, in particular of obesity-induced insulin resistance. In general, several intrinsic and extrinsic cellular mechanisms have been identified, which display a cause-effect relationship

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between weight gain and peripheral insulin resistance[5]. Intrinsic cell-signaling pathways include mitochondrial dysfunction, oxidative stress and endoplasmic reticulum (ER) stress, whereas alterations in adipokines and fatty acids levels and the presence of inflammation in metabolic tissue are the dominant extrinsic mechanisms that modulate insulin peripheral actions[5]. The purpose of the present review is focused on the role played by these mechanisms in its development. To this end, the molecular mechanisms of insulin signaling, regulation and resistance will be first addressed, and then, molecular aspects of inflammation, ER stress and mitochondrial dysfunction and their relationship with resistance will be reviewed.

Insulin actions

Insulin directly or indirectly affects the function of practically all body tissues, by eliciting a notorious variety of biological responses. Its metabolic actions on the liver, muscle and adipose tissue are the subject of intense global research, since these tissues are responsible for body metabolism and energy storage, and carry out important functions in the development of insulin resistance, obesity and DM2. Insulin is the main responsible for controlling cell nutrients uptake, usage and storage; it increases blood sugar absorption, mainly in muscular and adipose tissues, where it promotes its conversion into glycogen and triglycerides, respectively, while inhibiting its degradation. In addition, in the liver, it inhibits gluconeogenesis, glycogenolysis and ketogenesis, and promotes protein synthesis, mainly in muscular tissue. These actions are carried out thanks to a combination of rapid effects, such as glucose transport stimulation in adipose and muscle cells and regulation of the activity of key enzymes in metabolism, and of mechanisms that imply gene expression changes on the long-term[6,7].

Within cardiovascular physiology, insulin plays a key role in cardiac contractility, vascular tone and lipid, glucose and protein metabolism regulation[8,9]. One of its main functions is endothelial nitric oxide synthase enzyme (eNOS) activation, which leads to nitric oxide (NO) production in the vascular endothelium[10,11]. Insulin-induced NO production in the endothelium diffuses into both the lumen and vascular smooth muscle cells, where it activates the guanylate cyclase enzyme to increase GMPc levels, which induces vascular relaxation. Thus, increased blood flow through the action of insulin induces an increase in the use of glucose in target tissues[8,9]. Insulin also regulates glucose transport in cardiomyocytes, mainly through glucose transporter type 4 (GLUT-4), glycolysis, glycogen synthesis, lipid metabolism, protein synthesis, growth, contractility and apoptosis[8,9].

Insulin also has relevant functions in the CNS. Its presence in the brain was first detected by Havrankova et al.[12], who discovered high levels of insulin not only in humans, but also in various animal models[13]. Insulin plays a highly important neuromodulator role and insulin receptors and associated signaling pathways have been identified in different brain regions, where they regulate different physiological effects such as neuronal development, glucose metabolism, body weight and eating behaviors; it also participates in cognitive processes such as attention, learning and memory[14].

Molecular mechanisms of insulin action

Insulin is a 51-amino acid peptide, produced and secreted by pancreatic islets' β cells. It consists of two polypeptide chains, A and B, of 21 and 30 amino acids, respectively, which are connected by disulfide bridges[4,12,16]. Its biological actions begin when it binds to its receptor, an integral membrane glycoprotein, which is formed by two α-subunits and two β-subunits. The α-subunit, of 135 kDa, which contains the insulin binding site, is completely extracellular and binds to β-subunit extracellular region, as well as to the other α-subunit, through disulfide bridges. Each β-subunit, of 95 kDa, is composed of an extracellular domain, a transmembrane domain and an intracellular tyrosine kinase domain, which is activated by autophosphorylation[17].

The insulin receptor belongs to the family of receptors with tyrosine kinase (Tyr) intrinsic activity. Insulin binding to α-subunit of the receptor generates conformational changes that induce its catalytic activation and autophosphorylation of several Tyr residues located at β-subunit cytosolic region[17,18]. Autophosphorylated residues are then recognized by different adaptor proteins, which include members of the family of the insulin receptor substrate (IRS), out of which IRS-1 and IRS-2 are the two main substrates and most common intermediaries in insulin signal propagation initial stage. IRS acts as an adaptor molecule that organizes the formation of molecular complexes and triggers intracellular signaling cascades[19,20].

Most insulin actions are carried out by activation of two main signaling pathways: the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, also known as protein kinase B (PKB), responsible for most its metabolic actions, and the mitogen-activated protein kinases/Ras pathway (MAPK/Ras), which regulates gene expression and insulin-associated mitogenic effects (Fig. 1)[21].

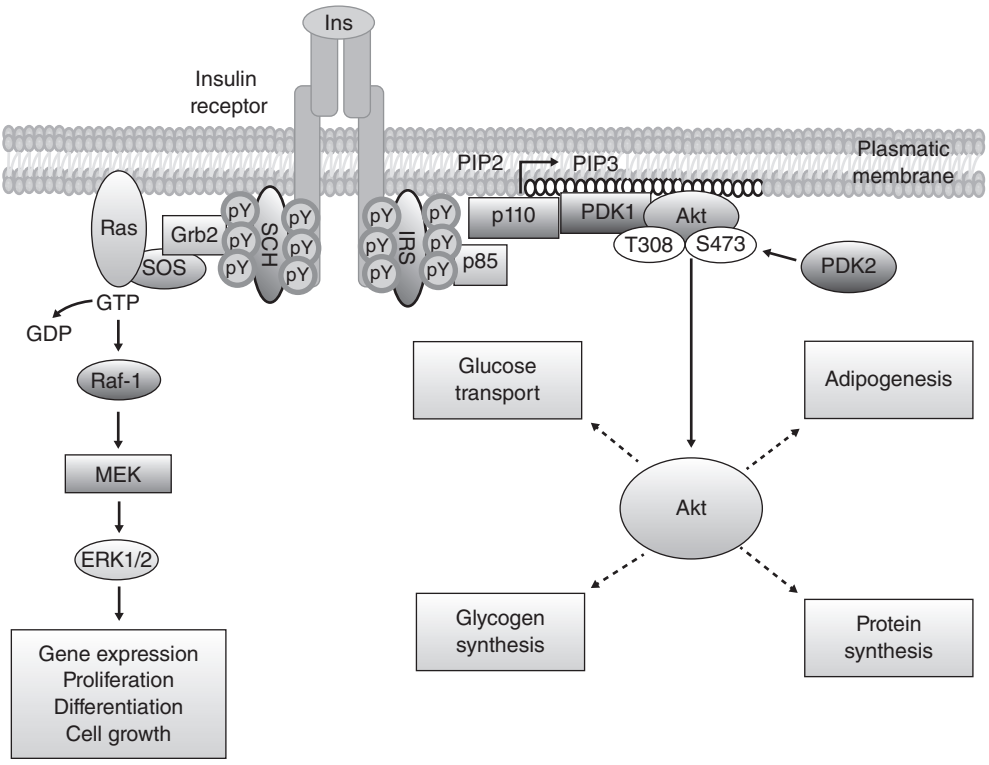

Figure 1. *Insulin signaling pathways. After interacting with its receptor, this recruits and phosphorylates mainly two adapting proteins: IRS, principal mediator of insulin metabolic actions, and SHC, which mediates cell proliferation and growth actions. Main IRS-mediated pathways include the PI3K/Akt pathway, which plays a central role in activation and regulation of several metabolic processes, including glucose transport stimulation, glycogen and protein synthesis and adipogenesis. In the case of SHC, it is associated with MAP kinases pathway activation to regulate its proliferative and growth functions.*

In the case of the PI3K/Akt pathway, the Akt kinase plays a central role in insulin signaling, since its activation leads to phosphorylation of an important number of substrates with key functions in a wide variety of biological processes, including enzymes, transcription factors, cell-cycle regulating proteins and apoptosis and survival proteins[22]. To date, three Akt isoforms have been identified (Akt 1, 2 and 3), out of which Akt2 appears to play an important role in insulin metabolic actions, including muscle and adipose tissue glucose uptake through GLUT-4 translocation from intracellular compartments to the cell membrane, to increase glucose uptake. Additionally, Akt participates in the synthesis of glycogen through GSK-3β inhibition, synthesis of proteins via mammalian target of rapamycin/ribosomal protein S6 kinase, of 70 kDa (kilodaltons), and synthesis of lipids (Fig. 1)[22,23].

On the other hand, insulin is known to be a potent growth factor; its growth-promoting effects are mediated by MAP/Ras pathway activation[24,25]. Activation of this pathway involves Tyr phosphorylation of IRS proteins and/or SH2 domain-containing protein (SHC), both of which, in turn, interact with growth factor receptor-binding protein 2 (Grb2), which recruits Sons of Sevenless (SOS) guanine nucleotide exchange factor to the plasmatic membrane for small G protein Ras activation, catalyzing the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) in Ras, which enables its activation. Ras-GTP operates as a molecular "switch", stimulating the MAPK cascade through Raf, MEK and ERK1/2 sequential activation[24,26]. Once active, ERK1/2 translocate to the nucleus and catalyze the phosphorylation of transcription factors that regulate gene expression and promote cell growth, proliferation and differentiation (Fig. 1)[24,27]. Interestingly, MAPK pathway inhibition by using dominant negatives or pharmacological inhibitors prevents insulin-mediated growth-promoting effects stimulation without its metabolic actions being affected[28]. However, one study carried out by Bost et al. demonstrated that ERK1 kinase is necessary for adipogenesis, which suggests participation of this pathway in insulin metabolic actions[25,29].

Insulin signal regulation

Insulin metabolic and growth-promoting actions are accurately regulated through self-regulation mechanisms

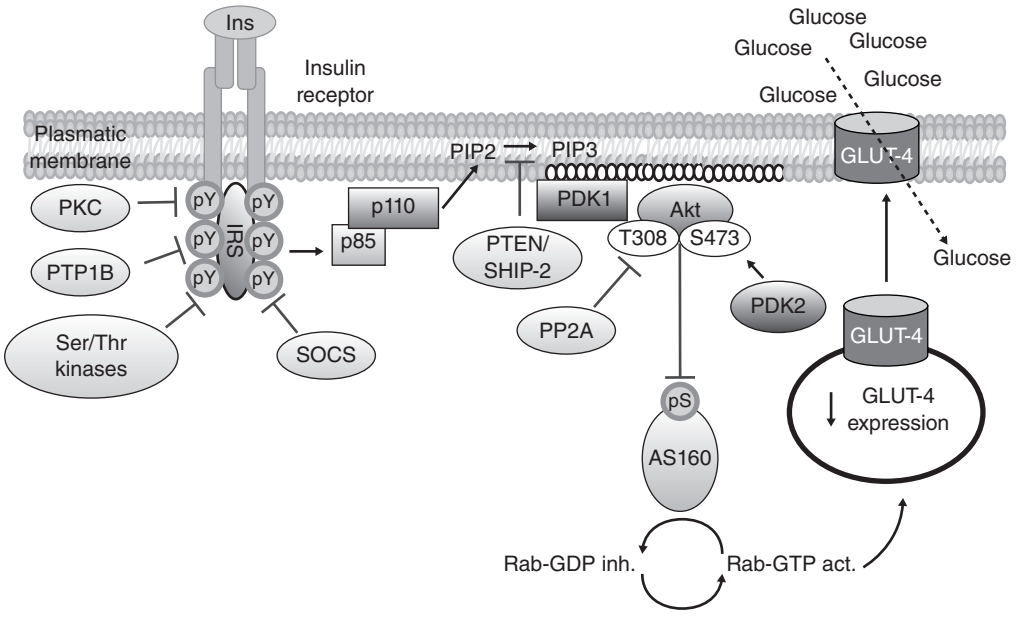

Figure 2. *Insulin actions' regulation. Insulin actions are highly regulated in order to promote adequate functioning of its metabolic, growth-promoting and cell proliferation actions. At the receptor level, several regulatory mechanisms have been described, including endocytosis and recycling; dephosphorylation of Tyr key residues that participate in receptor activation and association with adapting proteins, by PTP-1B action, and receptor phosphorylation on Ser/Thr residues by PKC and other Ser/Thr kinases, which affects insulin receptor enzymatic activity. These mechanisms alter receptor activity by disarranging protein complexes formation and regulating their number and cell location. There are other receptor-downstream insulin signaling regulation checkpoints: at the level of IRS proteins, by Ser/Thr residues phosphorylation and by SOCS action; at the Akt level, by phosphatase PP2A action and, at the level of PIP3 synthesis, by PTEN and SHIP-2 lipid phosphatase action, which specifically antagonize PI3K/Akt signalling. Grey arrows and lines indicate negative regulation pathways.*

(homologous regulation), where enzymes activated by the pathway itself inhibit insulin key signaling proteins activity[4,30]. In addition, there are homeostatic molecular mechanisms, unrelated to those activated by insulin, which can also inhibit this hormone's signaling (heterologous regulation)[4,30]. Both mechanisms are highly important, since they maintain cell homeostasis state, thus defining signal duration and range, as well as insulin actions[31].

Different homeostatic regulatory mechanisms have been identified at the receptor level, at IRS and in proteins located downstream of both, including PI3K, Akt or GLUT-4 (Fig. 2).

Several studies have demonstrated that insulin receptor activity is regulated by phosphotyrosine phosphatases action, which dephosphorylate specific Tyr-residues of the residues active receptor, thereby reducing its activity. In particular, there is evidence that phosphotyrosine phosphatase 1 B (PTP-1B) is an essential component of insulin actions regulating mechanisms[32-34]. PTP-1B studies carried out with knock-out mice provide evidence of the role of this phosphatase, since these animals show both increased insulin sensitivity and enhanced receptor Tyr phosphorylation, and are resistant to the development of obesity and insulin resistance induced by high-fat diet[32-34]. Conversely, PTP-1B overexpression in the pancreatic β cell line INS-1 decreased both receptor and IRS-1 insulin-stimulated Tyr phosphorylation, Akt phosphorylation and glucose-stimulated insulin secretion[35].

Another molecular mechanism associated with insulin receptor regulation is the phosphorylation of the β-subunit on Ser/Thr residues. There is evidence indicating that this phosphorylation affects receptor kinase activity in response to insulin binding, an alteration that has been observed in states of resistance and obesity both in rodents and in humans. The main receptor phosphorylation-associated kinase is protein kinase C (PKC), which phosphorylates it in different intracellular regions of β-subunit[36]. However, it has also been reported that other Ser/Thr kinases phosphorylate the insulin receptor and decrease its activity, such as protein kinase A (PKA), c-Jun amino-terminal kinase (JNK) and p38-kDa mitogen-activated protein kinase[32,36]. Among Ser/Thr possible phosphorylation sites, several are found close to autophosphorylation sites or within the catalytic domain, which might affect receptor conformation or access to Tyr residues[36].

Regulation at the level of insulin receptor expression represents another regulating mechanism of insulin actions. In the presence of insulin, Akt phosphorylates the transcriptional factor FoxO1 in at least three residues, which facilitates its interaction with protein 14-3-3. This interaction promotes FoxO1 exclusion from the cell nucleus and its eventual ubiquitination-dependent proteasomal degradation, thus preventing the transcription of the insulin receptor gene. Conversely, in the absence of insulin, such as in fasting periods, forkhead box O1 (FoxO1) transcriptional factor binds to the promoter region of the insulin receptor gene, thus stimulating its transcription[36-38].

Regarding the regulation of the IRS, its phosphorylation in Ser/Thr residues has been considered one of the main mechanisms of both homologous and heterologous regulation of insulin signal. Of the 230 IRS-located Ser/Thr residues, more than 70 potential phosphorylation sites for different kinases have been identified, including JNK, mTOR, ERK1/2, SIK-2 and different PKC isoforms[39]. There is experimental evidence that phosphorylation of multiple Ser/Thr residues of IRS represents a key mechanism in insulin signaling inhibition, both by physiological and pathophysiological activation. Several studies have demonstrated that phosphorylation of these residues is associated with insulin signal attenuation, since the tyrosine phosphorylation of IRS is altered, PI3K activity is decreased and its degradation is promoted[25,39].

Moreover, different adapting proteins have been identified that, when interacting with insulin receptor or with IRS, decrease their activity. For example, suppressor of cytokine signaling (SOCS) proteins, specifically SOCS-1 and SOCS-3, are potent repressors of insulin signaling pathway, whose expression is induced by insulin action on different tissues and cell lines[25,40,41]. SOCS have been proposed to regulate insulin signal by directly interacting with both insulin receptor and IRS, when both are active[24,41]. The insulin receptor-IRS/SOCS interaction inhibits Tyr phosphorylation of IRS by competing for the same interaction site in the insulin receptor, promotes IRS proteasomal degradation and inhibits insulin receptor kinase activity[25,41]. Grb10 and Grb14 are cytoplasmic adapting proteins that directly bind to insulin receptor phosphotyrosines (in the activation loop) through Src homology-2 (SH2) domains; this interaction decreases catalytic activity of the receptor and prevents its interaction with IRS. Expression of both proteins in adipose or muscular cells under conditions of obesity has been shown to decrease insulin sensitivity[42,43].

In addition to regulation at the level of the insulin receptor and IRS, there are regulation points below these proteins that also influence on insulin signal modulation. In this context, lipid phosphatases can regulate insulin signaling by modulating phosphatidylinositol-3,4,5-trisphosphate (PIP3) levels, which are generated by PI3K action. Phosphatase and tensin homolog (PTEN) dephosphorylates PIP3, thereby specifically antagonizing PI3K/Akt signaling[44,45]. Quite interestingly, a recent study by Shi et al.[46] has demonstrated that, in addition to decreasing PIP3 levels, PTEN can also dephosphorylate IRS-1, thus altering insulin signaling through the PI3K/Akt pathway by both these mechanisms[46]. Moreover, the SH2-domain containing inositol 5-phosphatase 2 (SHIP-2), dephosphorylates PIP3 as well and plays an important role in insulin signal regulation[47,48].

Molecular mechanisms of insulin resistance

A central characteristic of DM2 is insulin resistance, a condition where cells fail to adequately respond to insulin[32]. This insulin deficient signaling is caused by different alterations, including mutations and/or post-translational modifications in the insulin receptor, IRS or in downstream-located effector molecules. Most common insulin resistance alterations include a decrease in the number of insulin receptors and of their catalytic activity, an increased Ser/Thr phosphorylation state in insulin receptor and IRS, an increase in Tyr phosphatase activity, mainly PTP-1B, which participate in receptor and IRS dephosphorylation, a decrease in PI3K and Akt kinases activity, and defects in GLUT-4 expression and function[25]. These alterations reduce glucose uptake in muscular and adipose tissues and promote alterations at the metabolic level.

An essential factor contributing to insulin resistance is Ser/Thr hyperphosphorylation of IRS proteins. IRS hyperphosphorylation decreases its phosphorylation in Tyr and reduces its interaction with PI3K, thus altering Akt kinase phosphorylation and activation. Additionally, IRS phosphorylation on Ser/Thr residues has been reported to accelerate its degradation. Different agents, such as pro-inflammatory cytokines, saturated fatty acids (SFA), amino acids, endothelin 1, angiotensin II (Ang II) and states if hyperinsulinemia[49-51], increase the activity of kinases, such as several PKC isoforms, JNK stress kinase, mTOR, 70-kDa S6 ribosomal protein kinase, PKA and MAPK, which phosphorylate IRS[4].

The importance of increased phosphorylation status of IRS proteins has been documented in clinical trials with obese patients, where IRS-1 expression decreases by about 54%; this increase in degradation may be generated by a phosphorylation increase in Ser/Thr residues[4]. On the other hand, biochemical and genetic evidence indicates that Ser/Thr hyperphosphorylation throughout IRS-1 structure can reduce insulin-stimulated phosphorylation in Tyr by up to 50%. This level of inhibition is sufficient to cause glucose intolerance progressing to DM2, especially if pancreatic β cells fail to provide adequate compensatory hyperinsulinemia[53]. On the other hand, hyperinsulinemia itself can aggravate Ser/Thr phosphorylation in IRS-1 by activating the PI3K/Akt, PKC-τ/-λ or mTORC1/p70S6k pathways, which participate in insulin signal regulation.

Moreover, insulin signal regulating mechanisms have been identified at the level of Akt. In this sense, the production of ceramides by an increase in SFA metabolism, such as palmitate, can regulate Akt activity by directly modulating phosphoprotein phosphatase 2A (PP2A) activity, which dephosphorylates and inactivates it[54,55], and protein kinase C ζ (PKC-ζ) activity, which phosphorylates it in Ser[34] and inhibits its translocation to the membrane in order to be activated[56-58].

Inflammation and insulin resistance

Inflammation, which is a physiologic response for body protection, occurs in order to control physical, chemical or biological aggressions, and is characterized by an elevated number of white blood cells (WBC) and/or an increase in circulating or tissue pro-inflammatory cytokines levels[59]. There is experimental and clinical evidence indicating that obesity induces adipose, hepatic and muscular tissue alterations that entail a low-grade chronic inflammatory response, which contributes to insulin resistance and systemic metabolic dysfunction. Obesity is defined as the presence of an excessive amount of body fat or adipose tissue, which manifests itself as body weight increase associated with a higher distribution of visceral adipose tissue[25]. In patients with obesity, an elevated level of inflammatory markers has been detected, as well as a correlation between these markers and the presence of abdominal adiposity[60,61].

In the obesity state there is an increase in lipid accumulation, particularly in the adipose tissue, which elicits an increase in adipose cells size, adipose tissue expansion and altered secretion of adipokines and pro-inflammatory cytokines, as well as free fatty acids (FFA) aberrant release. FFA and pro-inflammatory cytokines act on metabolic tissues, such as hepatic and muscular tissues, thereby modifying the inflammatory response, as well as lipid metabolism, therefore contributing to metabolic syndrome. Furthermore, obesity has been shown to increase adipose tissue macrophage infiltration, which substantially contributes to cytokine production and secretion in response to obesity[62,63].

Pro-inflammatory cytokines secreted in the adipose tissue and by macrophages include resistin, tumor necrosis factor α (TNF-α), interleukins (IL) 6, 18 and 1β, monocyte chemotactic protein 1 and Ang II[64-66]. These factors, on one hand, contribute to local and generalized obesity-associated inflammation state and, on the other, as in the case of TNF-α, IL-6, IL-18, IL-1β and Ang II, can directly induce insulin resistance[25,67-69]. The most interesting of these findings, is that the inflammatory process contribution to adipose tissue insulin resistance is not only local, but also systemic. In the case of cytokines such as TNF-α, IL-6 and IL-1β, they induce insulin resistance by multiple mechanisms, such as Ser/Thr kinases activation, decrease in IRS-1, GLUT-4 and peroxisome proliferator-activated receptor gamma expression or SOCS-3 expression and activation (Fig. 3)[32,70-73].

Another important factor in obesity-associated inflammation is toll-like receptors (TLR) activation, in particular TLR-2 and TLR-4[32]. TLR is a family of receptors that belong to the innate immune system, which are generally activated by molecular patterns associated with pathogens such as lipopolysaccharide (LPS) and that induce inflammation through nuclear factor κB (NF-κB) pathway. Although TLRs are ubiquitously expressed, TLR-4 expression has been observed to be elevated in muscular and adipose tissue under obesity conditions. An interesting finding indicates that SFA are agonists for TLR-4, which suggests a potential role of these receptors in obesity-induced low grade inflammation. In this context, studies in mice with decreased expression of TLR-2 and TLR-4 signaling proteins show that these animals are protected against the development of obesity, insulin resistance and metabolic syndrome induced by high-fat diet[74,75]. To date, different mechanisms have been described by means of which TLR-4, when being activated by fatty acids, regulate insulin signal. For example, there is evidence that TLR-4 activation promotes IRS direct phosphorylation on Ser/Thr residues through JNK, NF-κ-inhibitor kinase (IKK) and MAPK activation[74,76]. TLR-4/NF-κB pathway activation in macrophages by FFA action, has also been reported to promote the synthesis and secretion of cytokines

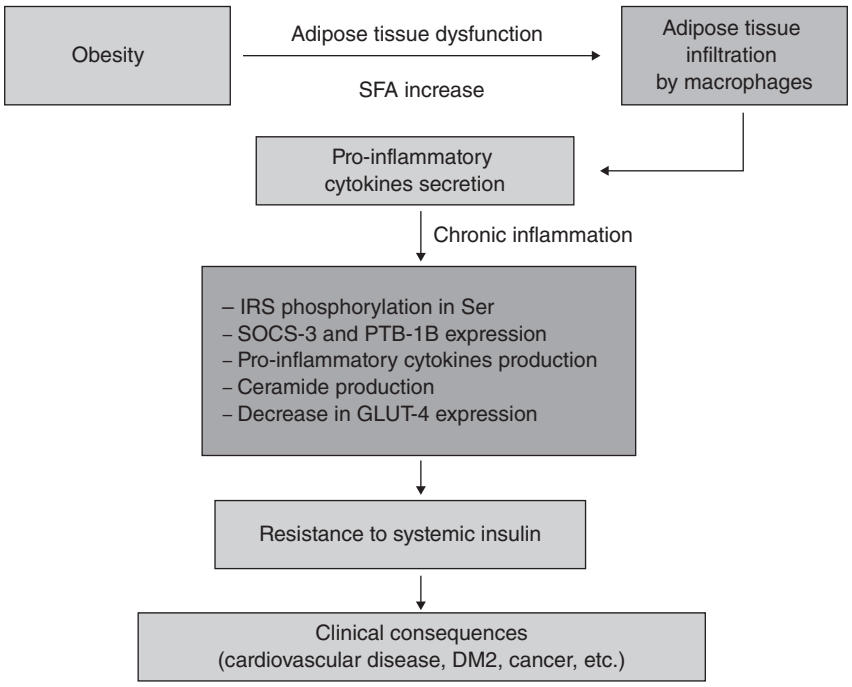

Figure 3. *Inflammation and insulin resistance. Obesity promotes a state of low-grade chronic inflammation, due to an increase in the number of macrophages infiltrating the adipose tissue, which promotes the secretion of inflammation mediators. On one hand, these factors contribute to a local and generalized obesity-associated inflammation state, and on the other, they can directly induce insulin resistance by promoting IRS's Ser residues phosphorylation, SOCS-3 expression, pro-inflammatory cytokines and ceramides production and a decrease in the expression of GLUT-4 and glucose transport. Insulin resistance is associated with the development of cardiovascular disease, DM2 and cancer, among other diseases.*

such as IL-6, TNF-α, IL-1β and IL-18, which contribute to adipose tissue inflammatory state during obesity[77,78]. The expression of SOCS-3 and PTP-1B, which are considered to be insulin signaling negative regulators, is also induced by TLR-4 activation[78]. Finally, the findings described by Holland et al.[79], demonstrated that, regardless of their role as ceramide precursors, SFA, through TLR-4 activation, increase the expression of enzymes that participate in ceramide synthesis. The increase in ceramide intracellular levels leads to PP2A phosphatase activation, which dephosphorylates and inactivates Akt, thus inhibiting the insulin signal[57]. Together, these data reinforce the idea that the immune system plays a crucial role in the development of insulin resistance and DM2.

ER stress and insulin resistance

In the endoplasmic reticulum (ER), important cellular functions take place, such as intracellular calcium storage, protein assembly and folding and post-translational modifications. In cellular stress conditions, which increase ER demand and entail an overload of its functional capacity, alterations are generated in its function and in the decrease of protein transport to the Golgi apparatus, misfolded protein expression and calcium depletion of this reservoir, which as a whole are called "reticulum stress"[80-82]. As a compensatory mechanism to ER stress, and specifically to misfolded proteins, a mechanism known as unfolding protein response (UPR) is activated in the organelle itself, which allows for ER functions homeostasis to be reestablished by means of protein synthesis inhibition and increase of both ER proteins degradation and chaperone levels to aid in protein folding[83]. If these adapting mechanisms are insufficient to restore ER homeostasis, the cell undergoes programmed cell death[84,85]. UPR includes activation of three stress sensor kinases: protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring kinase/endoribonuclease 1 (IRE-1) and activating transcription factor 6 (ATF-6), which detect protein misfolding and trigger UPR. UPR-generated responses include an increase in the levels of chaperons and folding enzymes that help in protein folding and prevent non-folded or misfolded proteins aggregation[86].

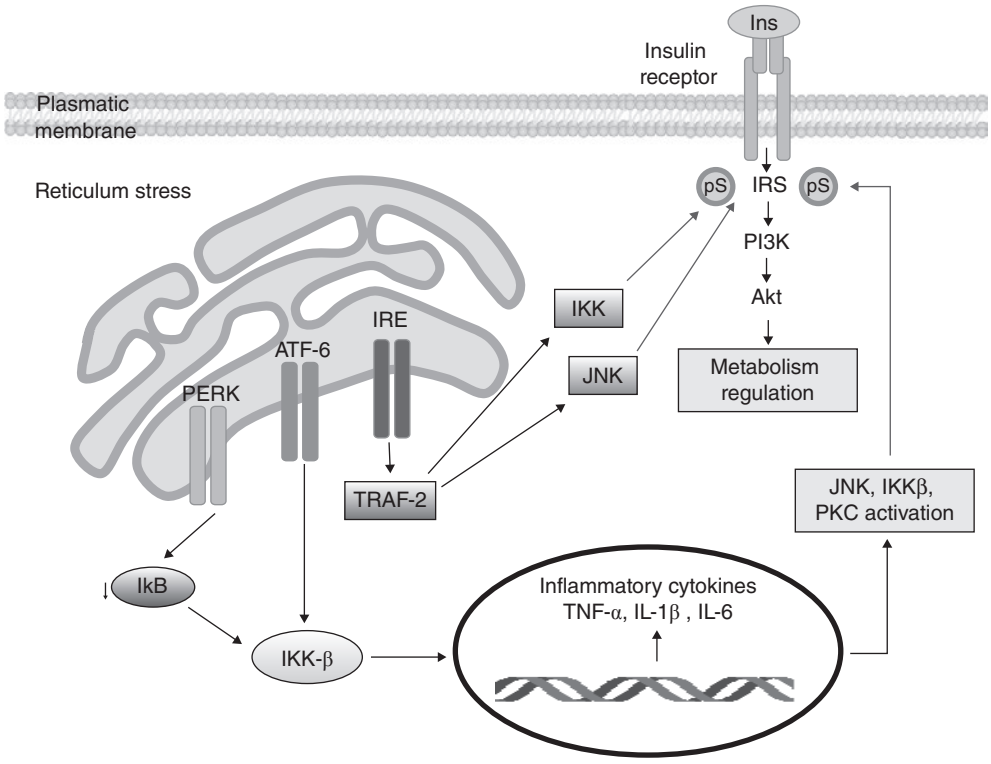

Figure 4. *Reticulum stress and its association with the development of insulin resistance. In response to protein misfolding, activation of three stress-sensor kinases is induced in the ER: PERK, IRE-1 and ATF-6, which detect protein misfolding and trigger a mechanism known as UPR. These kinases also promote mechanisms leading to the synthesis of inflammatory cytokines or activation of kinases that regulate IRS activation. PERK promotes the release of NF-κB from its inhibitor IκB, which causes its translocation to the nucleus and stimulates the synthesis of inflammatory cytokines such as TNF-α, IL-1β and IL-6. As a consequence, different Ser kinases are activated, such as JNK and PKC-θ, which leads to IRS-1 phosphorylation on Ser, which produces insulin resistance. The IRE-1 kinase phosphorylates and activates JNK, which in turn phosphorylates IRS-1 on Ser, an effect that reduces its capacity to interact with other signaling proteins, with the PI3K/Akt and MAP kinases pathways mainly being altered, which leads to insulin resistance. The ATF-6 pathway activates NF-κB as well, which results in the same pro-inflammatory response. Gray arrows and lines indicate negative regulation pathways.*

Different studies have demonstrated a close relationship between ER stress, inflammatory response and insulin resistance. In this context, PERK activation promotes NF-κB activation, due to suppression of its NF-κB inhibitor (IκB) translation, which causes its translocation to the nucleus, where it promotes the expression of a variety of genes involved in inflammatory pathways such as TNF-α, IL-1β and IL-6[84,87,88], which activate Ser kinases such as JNK[89], IKK-β[90] and PKC-θ[91]. Activation of these kinases results in phosphorylation of the receptor or IRS-1 on Ser/Thr residues, which entails insulin resistance. In the case of the IRE kinase, the interaction with TNF-α receptor-associated factor 2 (TRAF-2) leads to IKK-β and JNK activation, which in turn phosphorylate IRS-1. This phosphorylation has been associated with a decrease in the Tyr phosphorylation state of IRS, which reduces its capability to interact with other signaling proteins, with the PI3K/Akt and MAP kinases pathways being mainly altered[82,86,92]. In turn, ATF-6 also enables NF-κB activation, which, as previously mentioned, plays an important role in inflammatory cytokines expression (Fig. 4).

Interestingly, several studies in animal models with obesity have demonstrated the existence of reticulum stress in the liver, in pancreatic β cells, in the brain and in adipose tissue[92-94]. Furthermore, this condition has been detected in adipose and hepatic tissues of humans with obesity[95,96], and it was reverted in ER stress indicators in patients undergoing weight loss by surgical procedure[96].

ER chaperones and folding enzymes, such as glucose-regulated proteins (GRP) 78 and 94, protein disulfide isomerase, calnexin and calreticulin, have as their main function to ensure an adequate assembly and to organize protein tertiary structure, in addition to avoiding non-folded or misfolded protein aggregation[97]. Chaperones are associated with the membrane or in ER lumen, and recently have aroused great interest

owing to their relationship with metabolic syndrome. In this context, the use of chaperones of protein or chemical nature in different animal models decreases ER stress, which reflects in an increase in insulin sensitivity[98-100]. For example, oxygen-regulated expression of 150-kDa chaperonin protein reduces hyperglycemia and insulin resistance state[93,100]. In turn, GRP-78 overexpression restores systemic insulin sensitivity and improves hepatic steastosis[99].

Chronic administration of chemical chaperones such as 4-phenylbutyrate (PBA) and tauroursodeoxyholic acid (TUDCA) to ob/ob obese mice is able to normalize hyperglycemia and reduce the insulin resistance state present in these animals[98]. In addition, PERK and IRE-1 activation is importantly decreased by the treatment with both chemical chaperones, although it can not be ruled out that this is the only action of these compounds[98]. In studies conducted in humans with obesity, TUDCA and PBA administration increased insulin sensitivity and decreased resistance state[101,102].

Recently, alterations in the sarco/endoplasmic reticulum Ca^{2+} (SERCA) pump levels of expression, the function of which is to remove calcium from cytosol and give it back to the ER, have been shown to be associated with the development of ER stress and subsequently with insulin resistance. Under conditions that reduce SERCA activity, a luminal calcium environment is produced, which entails reticulum chaperones low activity[103]. In 2010, Park et al. reported that, when compared with control mice, obese mice (ob/ob) hepatic tissue showed a substantial reduction in SERCA pump expression[104]. On the other hand, treatment of diabetic subjects with rosiglitazone, an anti-diabetic drug, member of the thiazolidinediones family, increased SERCA expression, thus restoring the pump expression reduction observed in diabetic patients with altered hyperglycemia[105]. All of these data suggest a relationship between the insulin resistance state and SERCA pump reduced levels. Together, these results indicate that ER dysfunction importantly contributes to chronic metabolic deterioration.

Mitochondrial dysfunction in insulin resistance and DM2

The mitochondria, a cell organelle that plays an important role in energy metabolism, is in charge to provide most part of the required energy in the form of adenosine triphosphate (ATP), which is produced from organic molecules that are oxidized in the presence of oxygen. In the past few years, alterations at the mitochondrial level have been proposed to be associated with insulin resistance, but the mechanisms by means of which mitochondrial damage or dysfunction lead to resistance are the subject of debate.

Although the definition of mitochondrial dysfunction is controversial between different authors, in general terms, it can refer to a decrease in substrate, including lipids and carbohydrates, oxidation as a result of oxidative phosphorylation general decrease. Associated with this function loss, a decrease in mitochondrial numbers can be also found. This way, mitochondrial dysfunction can be the result of a decrease in this organelle's biogenesis, a reduction of mitochondrial protein content and/or an activity decrease in enzymes participating in the oxidative process. All these changes would presumably be responsible for leading to a decrease in substrate oxidation. Since mitochondria are primary organelles for fatty acids and glucose oxidation and metabolism, mitochondrial function reduction can contribute to FFA and lipid accumulation, which favors the development of insulin resistance[106]. In the case of FFA accumulation, it is accompanied by an increase in diacylglycerol (DG) and ceramide levels, which inhibit the insulin signal, DG by promoting PKC activation, which phosphorylates and inhibits the insulin receptor, and ceramides by negatively regulating Akt activation. This way, DG and ceramide accumulation constitutes a possible link between mitochondrial dysfunction and insulin resistance (Fig. 5).

Controversially, recent evidence indicates that mitochondrial dysfunction is rather the result[107,108] than the cause of resistance itself[109]. Genetic manipulation of insulin signaling different components, such as IRS-1 and IRS-2 double knockout (KO) specifically in the liver[110,111] and skeletal muscle[112], in addition to generating mice with insulin resistance, marked glucose intolerance and DM2[111,112], has shown a decrease in the number of mitochondria, altered regulation of genes that participate in mitochondrial function control and biogenesis, and alterations at the level of oxidative phosphorylation and ATP production[110,112]. The effects observed in these works are probably due to the fact that, when insulin signal deregulation is generated by the absence of IRS-1 and IRS-2, transcriptional factor FoxO1, which participates in the regulation of genes that control functions such as glucose production and lipid metabolism, can no longer be regulated by Akt specific phosphorylation. This indicates that FoxO1 is active in KO mice and that possibly it is responsible for regulating genes that participate in mitochondrial dysfunction, since FoxO1

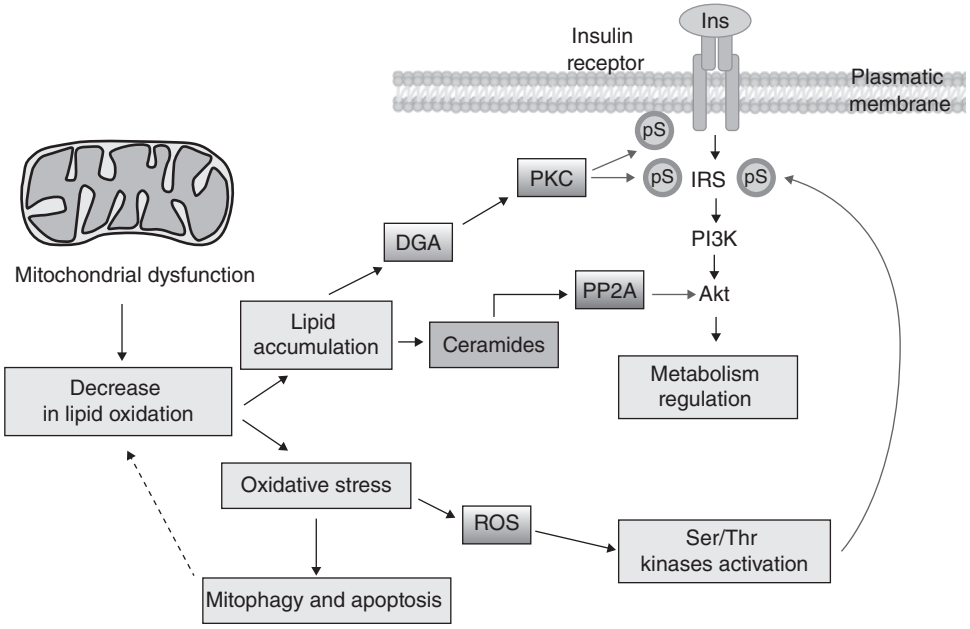

Figure 5. *Mitochondrial dysfunction and insulin resistance. Mitochondrial dysfunction promotes a decrease in lipid oxidation, as a result of oxidative phosphorylation general decrease; this contributes to a FFA and lipid accumulation that favors the development of insulin resistance. FFA accumulation is accompanied by an increase in DG and ceramide levels, which inhibit the insulin signal, DG by promoting PKC activation, which phosphorylates and inhibits insulin receptor, and ceramides by regulating Akt dephosphorylation and inactivation by PP2A. Lipid oxidation decrease also promotes the generation of ROS, which causes oxidative stress and several damages at the mitochondrial and cellular level, which potentially leads to mitophagy or, under high levels of stress, to apoptosis. Mitochondrial elimination by mitophagy might reduce their number, which would lead to a decrease in substrate oxidation and to ensuing lipid accumulation. ROS also cause Ser/Thr kinases activation, including IKK-β, JNK and PKC, which increase IRS proteins phosphorylation on Ser and subsequent insulin resistance.*

additional suppression (triple KO) has been able to rescue the metabolic phenotype and mitochondrial function[110]. These results suggest that the direct genetic resistance induction that occurs in these experimental models is able to drive to the development of mitochondrial dysfunction.

There is also evidence that free fatty acids (FFA) concentration increase during obesity, a condition that induces mitochondrial over-activation, promotes an increase in β-oxidation in order to increase energy availability, especially in the muscle, liver and brown fat. As a consequence, a large amount of ATP is generated by fatty acids catabolism if additional energy cannot be released as heat. When the ATP level exceeds the threshold, energy excess causes a negative feedback reaction to attenuate substrate-induced mitochondrial function. In this mechanism, ATP inactivates adenosine monophosphate (AMP)-activated protein kinase in order to reduce insulin-induced glucose uptake with the purpose to decrease ATP production[113]. Thus, insulin resistance represents a cell-protecting mechanism intended to control the response to ATP-induced stress in muscle and liver tissues. In this context, insulin signal-sensitizing agents are able to rescue tissues from insulin resistance by means of mitochondrial β-oxidation inhibition[59].

On the other hand, there is increasingly convincing experimental evidence that mitochondria-generated reactive oxygen species (ROS) play an important role in DM2 pathogenesis, progression and long-term complications. Decrease in substrate oxidation has been proposed to affect electron flow through their transport chain by eliciting their leak into oxygen and forming superoxide anions. The generation of these ROS causes different damages at the mitochondrial and cellular level, including mitochondrial DNA oxidative damage, protein aggregation and lipid peroxidation, which potentially leads to mitophagy (elimination of damaged mitochondria and cell-death prevention) or, under high levels of stress, to apoptosis. Mitochondrial elimination by mitophagy might reduce their numbers, which would entail a decrease in substrate oxidation and to an ensuing lipid accumulation. Furthermore, ROS formation has been reported to affect insulin release by pancreatic β cells and sensitivity to this hormone. On the other hand, ROS production is known to cause Ser/Thr

kinases activation, including IKK-β, JNK and PKC, which increase IRS proteins phosphorylation on Ser and subsequent insulin resistance (Fig. 5)[108].

In studies conducted both in humans and in animal models, hormones that contribute to insulin resistance, such as Ang II, have been shown to increase nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and ROS ensuing production, which is associated with mitochondrial structure and function abnormalities. The use of drugs that reduce Ang II actions, such as Ang II-converting enzyme inhibitors and AT_1-receptor antagonists, increases sensitivity to insulin, reduces ROS production and improves mitochondrial function and biogenesis[114-116].

Conclusions

Insulin carries out vital actions in energy metabolism and in cell proliferation and survival in peripheral and central tissues. The present review has addressed different aspects related to its function and signaling, as well as molecular mechanisms associated with the regulation of its actions. In addition, the role played by this hormone when alterations in its functioning occur has been brought to light. Insulin resistance, regarded as the most constant platform for metabolic syndrome and DM2 development, is a complex and multifactorial mechanism associated with multiple alterations at different levels of insulin signaling. Processes such as inflammation, reticulum stress and mitochondrial dysfunction are currently-identified key mechanisms associated with the development of insulin resistance. Therefore, better understanding of these pathways' molecular mechanisms and the existing interconnection between them and insulin resistance constitutes a current challenge for scientific research, since it can lead to the discovery of new therapeutic targets.

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