Molecular Mechanisms of Insulin Resistance in Chronic Kidney Disease

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Abstract

Insulin resistance refers to reduced sensitivity of organs to insulin-initiated biologic processes that result in metabolic defects. Insulin resistance is common in patients with end-stage renal disease but also occurs in patients with chronic kidney disease (CKD), even when the serum creatinine is minimally increased. Following insulin binding to its receptor, auto-phosphorylation of the insulin receptor is followed by kinase reactions that phosphorylate insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3-kinase (PI3K) and Akt. In fact, low levels of Akt phosphorylation (p-Akt) identifies the presence of the insulin resistance that leads to metabolic defects in insulin-initiated metabolism of glucose, lipids and muscle proteins. Besides CKD, other complex conditions (e.g., inflammation, oxidative stress, metabolic acidosis, aging and excess angiotensin II) reduce p-Akt resulting in insulin resistance. Insulin resistance in each of these conditions is due to activation of different, E3 ubiquitin ligases which specifically conjugate ubiquitin to IRS-1 marking it for degradation in the ubiquitin-proteasome system (UPS). Consequently, IRS-1 degradation suppresses insulin-induced intracellular signaling, causing insulin resistance. Understanding mechanisms of insulin resistance could lead to therapeutic strategies that improve the metabolism of patients with CKD.

Keywords

insulin resistance; chronic kidney disease; inflammation; cell signaling

INTRODUCTION

There is abundant evidence that insulin resistance complicates chronic kidney disease (CKD) by interfering with processes that contribute to abnormalities in the metabolism of lipids and carbohydrates and loss of protein stores [1-3]. Loss of protein stores is emphasized because muscle is a major site of insulin resistance and this loss is largely initiated by defects in the intracellular signaling pathway that is initiated by insulin [4]. Clinically, metabolic defects uncovered in rodent models of insulin resistance can be

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identified in patients with nearly normal kidney function as well as patients with end-stage renal disease (ESRD) [5;6]. Since insulin resistance is present in non-diabetic patients with minimal renal insufficiency, it is generally agreed that insulin resistance in CKD or complications of CKD cause insulin resistance; the development of insulin resistance is not limited to diabetic nephropathy or specific kidney diseases. Instead, renal insufficiency results in interference with the intracellular signaling pathway initiated by insulin. The consequences of insulin resistance have been intensively studied and recently, biochemical mechanisms causing insulin resistance have been elucidated. Because these mechanisms have been largely identified in animal models of CKD, they need to be corroborated in patients to determine how insulin resistance affects the metabolism of carbohydrates, lipid and protein.

The development of insulin resistance does not include defects in the binding of insulin to its receptor on cell membranes. Instead, insulin resistance results in "post-receptor defects" that interfere with intracellular signaling processes [7;8]. The defects in intracellular signaling result in several metabolic abnormalities. Understanding the development of insulin resistance is difficult because CKD and metabolic consequences of CKD (e.g., metabolic acidosis, increased glucocorticoid production, activation of inflammatory cytokines or excess angiotensin II) each cause insulin resistance and older patients seem to be more susceptible to developing complications of CKD [9-13]. Recent reports indicate that these different conditions share a common pathway that impairs insulin-initiated, intracellular signaling and hence, to the development of insulin resistance.

The intracellular signaling pathway that mediates the metabolic responses to insulin is well-defined. Normally, insulin binding to its receptor is followed by phosphorylation of specific tyrosines in the insulin receptor and insulin receptor substrated-1. This is followed by phosphorylation of phosphatidylinositol 3-kinase (PI3K) and the Akt kinase (p-Akt). In fact, the level of p-Akt in muscle are marker of impaired insulin signaling: a low level of p-Akt in muscle signifies impaired insulin-initiated intracellular signaling resulting in defects in the uptake and metabolism of glucose and lipids plus protein degradation in muscle. In contrast, high levels of p-Akt in muscle stimulate glucose uptake and the synthesis of proteins in muscles while suppressing the degradation of muscle proteins (Figure 1).

MEASUREMENTS OF INSULIN RESISTANCE

It is difficult to reproducibly assess insulin insensitivity or defects in insulin-stimulated intracellular signaling. Fliser et al. used an intravenous glucose tolerance test plus frequent measurements of blood glucose and insulin [5]. They compared non-diabetic patients with minimal to advanced loss of kidney function by infusing glucose intravenously and measuring levels of blood glucose and insulin over 2.5 hours. The group concluded that defects in insulin-mediated metabolism of glucose are present in patients with early stages of CKD and nearly normal serum creatinine levels. Recently, Jia et al., reported that CKD patients (median GFR of 46 ml/min per 1.73m^2) can have insulin insensitivity that is detected during a modified oral glucose tolerance test [14].

The "gold-standard" for documenting insulin resistance is the euglycemic clamp test: patients are given an intravenous infusion of insulin to stimulate glucose uptake in body tissues. At the same time, glucose is infused intravenously at a rate that maintains a constant level of blood glucose. If this rate is high, it indicates the presence of increased insulin sensitivity; a low rate of glucose infusion documents insulin resistance. The euglycemic clamp test has been used by DeFronzo et al., to demonstrate that insulin resistance is present in ESRD patients as well as in patients with complications of CKD such as metabolic acidosis ([6,13,15]). Unfortunately, the euglycemic clamp test is time-consuming and requires trained personnel so it is rarely used in clinical practice. Alternatively, the insulin suppression test can detect insulin resistance. It is based on infusing hormones/medications (i.e., octreotide, epinephrine, or propranolol) to suppress the release of endogenous insulin. At the same time, insulin and glucose are infused to achieve steady-state blood levels ([4,16]). The estimated insulin sensitivity in this test is inversely proportional to the steady-state blood glucose concentration (([4])). A third method of identifying insulin resistance and beta-cell function is based on fasting values of blood insulin and glucose, the homeostatic model of insulin resistance (HOMA-IR) ([4]). The technique is insufficiently sensitive to detect insulin resistance reliably in a small group of patients and it has been used mainly to assess insulin resistance in large numbers of subjects. The HOMA IR shortcoming is its reliance on static measurements of fasting plasma insulin and glucose. Reportedly, comparisons of HOMA IR and the euglycemic clamp technique reveal a reasonable correlation (i.e., r = 0.88) although other investigators report lower correlations (i.e., r ~ 0.6) ([17,18]). Additional details of tests of insulin resistance are provided by Pham et al. ([4]).

STIMULI CAUSING INSULIN RESISTANCE IN CKD

The genesis of insulin resistance has been studied intensively for two reasons: firstly, inflammation is often associated with insulin resistance and abnormal metabolism of protein, carbohydrate and lipids ([19,20]). Secondly, the presence of insulin resistance is associated with an increased risk of atherosclerosis even in patients with mild degrees of CKD. The atherosclerosis risk is linked to increased circulating monocytes and decreased high-density, lipoprotein cholesterol ([6,19,21-24]). To understand how inflammation is related to insulin resistance, investigators have examined biochemical responses of macrophages, adipocytes or skeletal muscle to determine how inflammation can stimulate the production of inflammatory cytokines and how the cytokines might interfere with the functions of insulin ([22,23,25-27]). For example, patients in the early stages of CKD exhibit high levels of circulating proinflammatory cytokines such as TNF-α, interleukin-6 (IL-6), interferon-γ (IFN-γ) and lipopolysacchride (LPS) ([23,28-30]). Although these circulating pro-inflammatory cytokines are produced by kidney, adipocytes, the liver or muscles, the triggers stimulating their production are not clear ([12,19,29]). There are at least two reasons to investigate how inflammation-stimulated defects affect metabolism. Firstly, circulating cytokines can interfere with biochemical pathways resulting in insulin resistance and its metabolic consequences; and secondly, CKD-induced increases in circulating cytokines are associated with a significant risk of cardiovascular disease and death ([5,22,28]).

How does chronic inflammation induced by CKD cause insulin resistance? We uncovered a novel pathway by which inflammatory mediators (e.g., TNF-α or Il-6) cause insulin

resistance ([22;31]). Initially, we used a RNA microarray analysis to examine potential mediators of insulin resistance in muscles of a mouse model of CKD. There was a 5.2-fold increase in the expression of a transmembrane glycoprotein, SIRP-α, in muscles of mice with CKD ([31]). SIRP-α was shown to interact directly with the insulin receptor in muscles of mice with CKD. The interaction reduced phosphorylation of tyrosines present in both the insulin receptor and IRS-1 and the decrease in tyrosine phosphorylation reduced insulin signaling in muscle cells. Since IRS-1 phosphorylation was also reduced, we concluded that a low value of IRS-1 caused insulin resistance by interrupting insulin-stimulated intracellular signaling. The trigger of increased SIRP-α expression in muscle is activation of the NF-κB pathway. When the NF-κB pathway was inhibited, there was a sharp decrease in SIRP-α expression plus increased tyrosine phosphorylation and activation of the insulin receptor and IRS-1. The responses were shown to be physiologically relevant because suppression of NF-κB sharply reduced SIRP-α expression. There also was inhibition of the proteolytic activity of the ubiquitin-proteasome system (UPS) preventing muscle protein losses ([31]).

Other triggers of insulin resistance include uremic toxins. For example, accumulation of p-cresyl sulfate in patients with CKD or in animal models of CKD resulted in impaired insulin-stimulated intracellular signaling ([32]). Furthermore, when normal mice were treated with p-cresyl sulfate, insulin resistance developed and lipids accumulated in muscle and liver ([32]). Specifically, p-cresyl sulfate administration resulted in an 83% increase ($p<0.001$) in the lipid content of skeletal muscles of mice and this response was principally derived from white adipose tissue. There also was a 23% ($p<0.05$) increase in the lipid content of livers ([32]). A similar ectopic redistribution of lipids developed in mice with CKD but no supplements of p-cresyl sulfate. Notably, the stimulus for developing insulin resistance in mice with CKD was the release of adipokines and cytokines from adipose tissues. In fact, it was found that redistribution of lipids to muscle increased reactive oxygen species (ROS) and activated serine/threonine kinases (JNK, IKK, P38 MAPK). These responses are relevant because each of these inflammatory factors can initiate insulin resistance ([33]). In cultured 3T3-L1 adipocytes or human adipocytes, addition of p-cresyl sulfate has been associated with suppression of lipogenesis and stimulation of lipolysis ([32]). Thus, increased levels of the uremic toxin, p-cresyl sulfate stimulate inflammation and ROS generation leading to changes in insulin-stimulated intracellular signaling and hence, insulin resistance ([33]).

An increase in circulating aldosterone occurring in patients with CKD produces a different mechanism of insulin resistance ([34;35]). There is an inverse relationship between an increasing level of circulating aldosterone and declining kidney function ([35]). In a prospective, randomized, placebo-controlled study evaluating non-diabetic patients with Stages 2-5 CKD, the efficacy of the aldosterone inhibitor, spironolactone, was evaluated. The trigger initiating an increase in mineralocorticoid receptor activation resulting in insulin resistance has been shown to be an increase in the accumulation of asymmetric dimethyl arginine (ADMA). This molecule was found to impair insulin signaling in adipose tissues of rodents with CKD and it accumulates in patients or mice with CKD. The insulin resistance associated with excess ADMA levels can be reversed by treating rodents or humans with spironolactone ([35]).

An excess of urea has also been implicated as a mechanism causing insulin resistance at least in cultured 3T3-L1 adipocytes ([36]). In adipocytes, it was found that adding a high concentration of urea stimulated the production of both ROS and O-linked beta-N-acetylglucosamine (O-GlcNAc). In mice, toxic responses to ROS and O-GlcNAc were largely eliminated by treatment with an antioxidant mimetic, SOD/catalase. Administration of the antioxidant also prevented the development of insulin resistance. These interesting examples of uremic toxin-generated insulin resistance plus the efficacy and safety of administering an antioxidant mimetic need to be evaluated in patients. Otherwise, the relationship of putative toxins to mechanisms that develop into metabolic abnormalities of CKD will remain speculative.

Metabolic acidosis is another consequence of CKD that has been associated with the development of insulin resistance ([15;37;38]). Although only relatively small groups of patients have been rigorously studied to discern responses to correcting acidosis, the responses to metabolic acidosis are of special interest because improving acidosis in patients with CKD can preserve their muscle mass while slowing the loss of kidney function ([39]). In CKD, metabolic acidosis develops not only because the ability to excrete acid is limited but also because excess acid is produced when patients with CKD eat protein-rich foods, especially, foods with increased sulfa-containing amino acids and phosphorylated proteins and lipids ([40;41]). Fortunately, metabolic acidosis can be corrected simply by administering supplemental $NaHCO_3$ or by changing the diet of patients with CKD to incorporate less meat and more vegetables and fruits ([38;39;41;42]). This same dietary-based approach can substantially lower blood pressure, thereby treating another complication of CKD ([43]).

An increase in circulating angiotensin II (Ang II) is another complication of CKD that causes insulin resistance. The mechanism for this association between Ang II and insulin resistance involves Ang II-stimulated production of IL-6 and Serum Amyloid A ([12;44]). These mediators and potentially other circulating inflammatory cytokines can stimulate the production of SOCS3 ([12]). As will be described, an increase in SOCS3 reduces the levels of IRS-1 which interferes with insulin-stimulated intracellular signaling. Thus, Ang II infusion causes insulin resistance because IRS-1 levels are low, interfering with insulin-stimulated intracellular signaling (Figure 1).

MECHANISMS OF INSULIN RESISTANCE

In this discussion, we will concentrate on identifying mechanisms that interfere with insulin signaling in skeletal muscles because this organ is the principal site of the insulin resistance caused by CKD. The breakthrough in identifying a common mechanism that could explain the development of insulin resistance in several conditions associated with CKD was the discovery that the ubiquitin-proteasome system (UPS) degrades proteins in muscle and other cells by a process that is remarkably specific ([45]). The common mechanism for the development of insulin resistance is that the UPS specifically degrades IRS-1, thereby interrupting the intracellular signaling pathway that is initiated by insulin (Figure 1). The loss of IRS-1 reduces p-Akt levels which results in abnormal metabolism of glucose, lipids and protein. Triggers of UPS activity initiate an integrated program for the degradation of an intracellular protein ([45;46]). The specificity of the degradation pathway arises because a

protein substrate destined for degradation in the UPS is initially tagged by ubiquitins conjugated to the protein substrate. This critical ubiquitin conjugation process is largely responsible for the specificity of proteins being degraded. Ubiquitin conjugation involves a series of ATP-dependent reactions. The initial reaction is an ATP-dependent activation of ubiquitin by an E1 ubiquitin activating enzyme. The activated ubiquitin is then transferred to an E2 ubiquitin-transfer protein. The third step is activation of a specific ubiquitin E3 ligase that interacts only with a specific substrate protein or family of proteins that are recognized by the ubiquitin E3 ligase. The ubiquitin E3 ligase repeats transferring activated ubiquitins to the substrate protein until there is a chain of 5 ubiquitins. At this stage, the substrate protein is "marked" for degradation ([45]). Subsequently, ubiquitin-conjugated substrate proteins are recognized by the 26S proteasome and using ATP, the 26S proteasome removes the ubiquitins and unwinds the substrate protein so it can be inserted into the central core of the 26S proteasome. In this central core, the protein is degraded to peptides which are released into the cell cytoplasm where they are degraded by peptidases (Figure 2).

These biochemical reactions provide a mechanism by which different conditions cause degradation of IRS-1 and hence, insulin resistance because the loss of IRS-1 interrupts intracellular signaling (Figure 1). How do CKD and the different complications of CKD cause degradation of IRS-1? It appears that each complicating disorders associated with insulin resistance stimulates the expression of a different E3 ubiquitin ligase. The key determinant of specificity is that each specific ubiquitin E3 ligases conjugates ubiquitin to IRS-1. Since ubiquitin conjugation to IRS-1 marks it for degradation in the 26S proteasome, it is the degradation of IRS-1 that results in insulin resistance. Evidence that activation of specific ubiquitin E3 ligases causes insulin resistance is summarized below.

SOCS1/SOCS3/Elongin BC

Insulin resistance frequently develops in patients who have physiological conditions associated with an increase in circulating proinflammatory cytokines ([47]). Proinflammatory cytokines such as IL-6 are associated with insulin resistance because the cytokines stimulate the expression of the SOCS3 protein which in turn accelerates the breakdown of IRS-1 in the UPS. Notably, there are eight members of the SOCS family of proteins (SOCS1 through SOCS7 plus CIS); these are distinguished by similarities in amino acid sequences plus the presence of a conserved SOCS box at the C-terminus of SOCS proteins ([48;49]). The interaction between SOCS proteins and proinflammatory cytokines is complicated because SOCS proteins also suppress inflammatory pathways by a mechanism involving binding of the SH2 domains in SOCS proteins to cytokine receptors ([50;51]). A special function of certain SOCS proteins (SOCS1, SOCS3, or SOCS6) is that they inhibit insulin signaling by promoting the degradation of IRS-1 in the UPS ([49;52]). Thus, overexpression of SOCS1 was found to reduce the levels of IRS-1 and IRS-2 proteins in muscle while elimination of SOCS1 substantially increases insulin sensitivity ([53]). For example, we have found a link between a constant infusion of Ang II into mice and an increase in SOCS3; mice infused with Ang II developed higher circulating levels of IL-6 plus an increase in the production of SOCS3. These responses result in insulin resistance because IRS-1 and p-Akt are both reduced in muscles ([12]). The mechanism by which SOCS3 or SOCS1 reduces IRS-1 levels in muscles requires expression of an E3 ubiquitin ligase consisting of Elongin BC and Cullin

proteins ([54]). Evidence that this mechanism results in insulin resistance is that mutation of the Elongin BC binding motif in SOCS1 prevents the conjugation of ubiquitin to IRS-1 or IRS-2 and this reaction in turn suppresses the degradation of IRS-1 and IRS-2. As expected, deletion of Elongin BC has been shown to prevent the development of insulin resistance ([55;56]). It is tempting to speculate that the SOCS-mediated degradation of IRS proteins could be a general mechanism for the insulin resistance that is induced by inflammation ([53]).

Casitas B-lineage lymphoma b (Cblb)

Patients ingesting diets containing excess fat and/or glucose often develop insulin resistance. In mice, the mechanism for this association requires expression of the carbohydrate-responsive element binding protein (ChREBP) and the sterol regulatory element binding protein 1c (SREBP1c); their expression is stimulated by energy-rich diets. Both proteins in turn, stimulate myostatin production which leads to degradation of IRS-1 via a Smad3-dependent, up-regulation of the Cblb protein ([57]). The Cblb protein belongs to the Cbl family of proteins (e.g., Cblb, c-Cbl, and Cbl-c) and it exhibits properties of an E3 ubiquitin ligase ([58]). These Cbl proteins contain a tyrosine kinase binding domain plus a RING-finger domain which interact to facilitate E3 ubiquitin ligase activity. The mechanism for the association of these domains of Cblb proteins and the development of insulin resistance is that Cblb is an E3 ubiquitin ligase that targets IRS-1 for degradation ([59]). Notably, mice lacking Cblb or c-Cbl are protected from the development of adiposity and insulin resistance even when they are fed a high fat diet ([60-62]).

MG53 (or TRIM72)

MG53 is an E3 ubiquitin ligase that is activated in cultured C2C12 muscle cells through interaction with the E2 ubiquitin ligase, UBE2H. MG53 activity is relevant to the development of insulin resistance because MG53 stimulates UPS-mediated degradation of IRS-1 ([63]). Notably, MG53 is present in muscles of mice fed a high-fat diet (HFD), in db/db diabetic mice, in spontaneously hypertensive rats and in obese non-human primates that exhibit characteristics of the metabolic syndrome ([64]). MG53 expression rises in response to more than one physiological condition but each such condition stimulates the conjugation of ubiquitin to IRS-1 leading to IRS-1 degradation and the development of insulin resistance ([65]). As expected, genetic deletion of MG53 in mice actually ameliorates the development of insulin resistance in skeletal muscles and increases the level of IRS-1, even when the mice are fed a high-fat/high sucrose diet ([66]).

Fbxo40

It has been demonstrated that prolonged exposure of cells to growth factors such as insulin or IGF-1 causes the cells to become insulin resistant ([67]). The mechanism underlying the development of insulin resistance following prolonged exposure to growth factors depends on expression of the E3 ubiquitin ligase, Fbxo40. This enzyme stimulates ubiquitin conjugation to IRS-1 leading to IRS-1 degradation in the 26S proteasome. The result of these interactions is impaired glucose metabolism. For example, it was found that incubation of cultured, C2C12 muscle cells with IGF-1 increased the degradation of IRS-1. However, mutation of the Fbxo40 E3 ubiquitin ligase in cultured, C2C12 muscle cells resulted in an

increase in the half-life of IRS-1, even though the cells were continuously exposed to IGF-1. In contrast, when Fbxo40 was genetically deleted in mice, there was an increase in IRS-1 levels in skeletal muscles and muscle hypertrophy developed ([67]). We recently found that CKD activates the Stat3 transcription factor in muscle and this in turn stimulates the expression of Fbxo40 resulting in a lower level of IRS-1 in muscle and the development of insulin resistance. Notably, this sequence of reactions was reversed by treating mice with a small molecule inhibitor of Stat3 activation (unpublished results). These responses provide a mechanism for the development of insulin resistance in muscles of mice with CKD

Cullin7-Fbw8

A pathway similar to that of Fbxo40 is involved in the growth factor-induced insulin resistance that is mediated by Cullin7-Fbw8. When cells were chronically exposed to insulin or IGF-1, activation of either Cullin-Fbw8 or Fbxo40 resulted in insulin resistance because both of these specific, ubiquitin E3 ligases are capable of conjugating ubiquitin to IRS-1. The E3 ubiquitin ligase that mediates the Cullin7-Fbw8 protein pathway conjugates ubiquitin to IRS-1, especially when the IRS-1 has undergone serine phosphorylation ([68]). Following conjugation of ubiquitin to serine-phosphorylated IRS-1, there is degradation of IRS-1 in the UPS resulting in reduced p-Akt and the development of insulin resistance. The evidence for a physiological role of the Cullin7-Fbw8 E3 ubiquitin ligase includes the report that overexpression of Fbw8 alone or Cullin7-Fbw8 in different cells markedly reduces the steady-state level of IRS-1 and shortens its half-life. Likewise, overexpression of Cullin7 and Fbw8 suppresses the synthesis of muscle proteins that are mediated by activated mTOR and p70S6K ([69]). Alternatively, depletion of Cullin7 in cultured, C2C12 muscle cells has been shown to improve glucose metabolism. These biochemical responses document that the E3 ubiquitin ligase activity of Cullin7-Fbw8 proteins is involved in regulating insulin-stimulated intracellular signaling.

The clinical relevance of these biochemical mechanisms to the development of insulin resistance has not been extensively examined but there are hints that insulin resistance is associated with reduced levels of IRS-1. For example, Mashili et al., obtained muscle biopsies from obese subjects and patients with type 2 diabetes and uncovered increased expression of the Stat3 transcription factor and both Janus kinase 2 and SOCS3 ([70]). Since infusion of Ang II into mice, has been shown to increase SOCS3 expression in muscle while decreasing the level of IRS-1, the results of Mashili et al., are consistent with events present in animal models of insulin resistance([12]). It is time to examine whether the complex mechanisms regulating metabolism in cells or animal models also are present in humans.

CONCLUSIONS

Detailed investigations of animal models of insulin resistance in conditions as different as CKD, metabolic acidosis, the presence of uremic toxins, inflammation, excess Ang II and glucocorticoids suggest that responses to these varied conditions share a common mechanism for the development of insulin resistance. Evidence that a common pathway to insulin resistance arises because each of the conditions associated with CKD is characterized by expression of a specific ubiquitin E3 ligase which stimulates degradation of IRS-1 and

low levels of IRS-1 and p-Akt interfere with the intracellular signaling that regulates insulin-stimulated metabolism of glucose, lipids and protein. Specifically, the expression of an ubiquitin E3 ligase leads to conjugation of ubiquitin to the substrate protein, IRS-1 which is degraded by the 26S proteasome. Five examples of this mechanism explain how different physiological conditions can stimulate degradation of IRS-1 and the development of insulin resistance.

Acknowledgments

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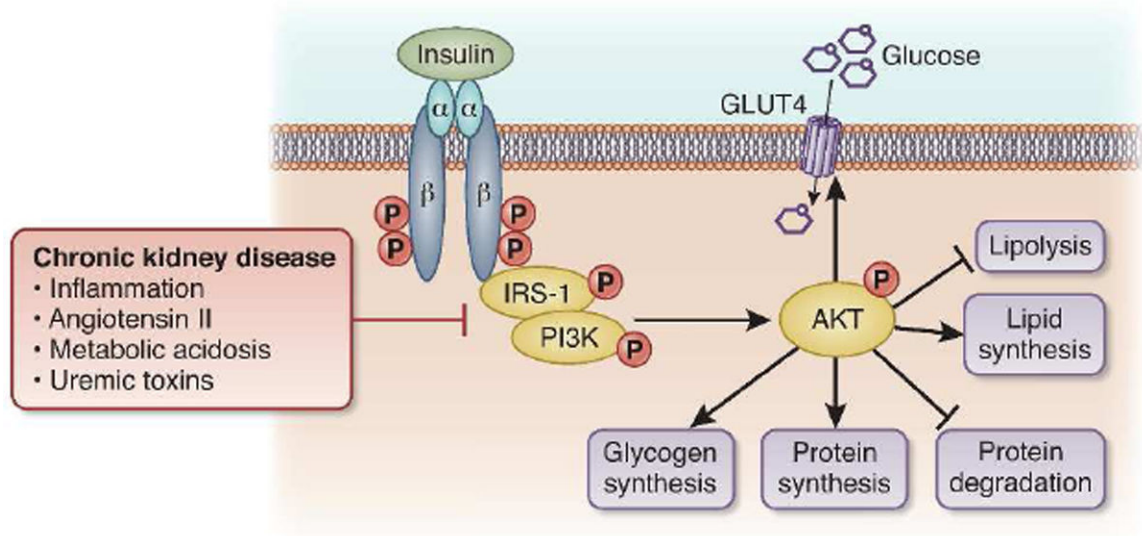
Figure 1.

is a pictorial representation of the intracellular insulin signaling pathway. When insulin binds to its receptor, the insulin receptor auto-phosphorylates tyrosines in the receptor. Subsequently, kinase activities phosphorylate IRS-1, PI3K and Akt which change metabolic processes including glucose uptake and lipid and protein metabolism. Interruption of this signaling pathway causes insulin resistance with impairment of insulin-stimulated metabolic functions. The insulin signaling pathway can be interrupted by chronic kidney disease inducing inflammation, excess angiotensin II, metabolic acidosis and several uremic toxins leading to defective metabolism of glucose and lipids plus reduced protein synthesis and increased protein degradation causing loss of muscle mass.
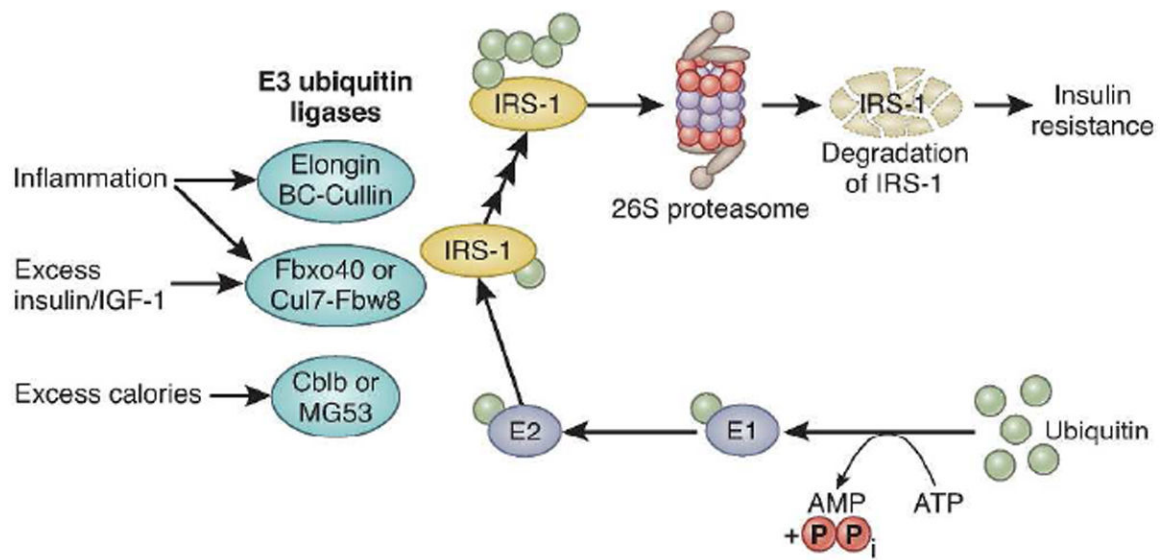
Figure 2.
is a scheme that results in a reduced level of IRS-1 due to its degradation by the ubiquitin-proteasome system (UPS). In the presence of ATP, the E1 ubiquitin activating enzyme stimulates ubiquitin leading to its interaction with an E2 ubiquitin transfer protein. Subsequently, IRS-1 reacts with a specific E3 ubiquitin ligase (Elongin BC-Cullin, Fbxo40 or Cul7-Fbw8, Cblb or MG53) that are activated by individual processes (inflammation, excess insulin/IGF-1, or excess calories respectively) which then selectively transfers activated ubiquitin from the E2 ubiquitin transfer protein to IRS-1. This process is repeated until a chain of at least 5 ubiquitins is attached to IRS-1. The chain is then recognized by the 26S proteasome which removes ubiquitins and unwinds IRS-1 so it can be injected into the central core of the 26S proteasome. IRS-1 is then degraded by the 26S proteasome causing insulin resistance.