Insulin resistance in chronic kidney disease: new lessons from experimental models

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ABSTRACT

Insulin resistance (IR) is a common feature of chronic kidney disease (CKD), but the underlying mechanisms still remain unclear. A growing body of evidence suggests that IR and its associated metabolic disorders are important contributors for the cardiovascular burden of these patients. In recent years, the modification of the intestinal flora and activation of inflammation pathways have been implicated in the pathogenesis of IR in obese and diabetic patients. All these pathways ultimately lead to lipid accumulation in ectopic sites and impair insulin signalling. These important discoveries have led to major advances in understanding the mechanisms of uraemia-induced IR. Indeed, recent studies show impairment of the intestinal barrier function and changes in the composition of the gut microbiome during CKD that can contribute to the prevailing inflammation, and the production and absorption of toxins generated from bacterial metabolism. The specific role of individual uraemic toxins in the pathogenesis of IR has been highlighted in rodents. Moreover, correcting some uraemia-associated factors by modulating the intestinal flora improves insulin sensitivity. This review outlines potential mechanisms by which important modifications of body homeostasis induced by the decline in kidney function can affect insulin sensitivity, and the relevance of recent advances in the field to provide novel therapeutic approaches to reduce IR associated cardiovascular mortality.

Keywords: chronic kidney disease, inflammation, insulin resistance, lipotoxicity, uraemic toxins

INTRODUCTION

Chronic kidney disease (CKD) is a risk factor for cardiovascular disease, and this increase in disease burden cannot be solely explained by traditional cardiovascular risk factors. In the 1980s, DeFronzo et al., using the ‘gold standard’ euglycemic hyperinsulinemic clamp technique, found evidence of insulin resistance (IR) in CKD patients [1]. They suggested that the site of this resistance lies in the binding of insulin to its receptor and can be reversed by dialysis. It is now well established that the decline of renal function is associated with the development of IR with impaired insulin-induced glucose utilization of peripheral target tissues. Since this seminal study, there has been a renewed interest in IR in CKD, especially as IR is an independent risk factor for cardiovascular morbidity and mortality in patients with CKD [2].

Although the underlying causes of IR in CKD remain unclear, understanding the mechanisms is pivotal. In obesity and diabetes, emerging studies suggest an interconnected network linking innate immunity and inflammation to metabolic diseases, and the major role of adipose tissue and intestinal flora in the control of energy metabolism and insulin sensitivity [3, 4]. During the last two decades, several cellular and animal models have enabled us to better understand the mechanisms underlying IR in CKD. The field has progressed rapidly with the availability of tools such as high-resolution mass spectrometry, 16S rRNA gene sequencing, metabolomic and metagenomic sequencing enabling even broader insights into the composition of uraemia retention molecules (URMs) and gut microbiota. There is strong evidence that there is an increased colonic generation or absorption of bacterial URMs in CKD [5–7]. Moreover, specific factors that are produced endogenously by tissue metabolism or intestinal bacteria have been shown to be involved in the uraemic disturbed insulin signalling pathways [8, 9]. Furthermore, white adipose tissue (WAT) dysfunction is now considered to be an important source of molecules that are responsible, at least in part, for the metabolic disturbances in these patients [10]. All these pathways are closely linked to changes in fatty acid uptake, lipogenesis and energy metabolism, which can lead to ectopic lipid accumulation in visceral tissues and impair insulin signalling through inhibitory serine phosphorylation of insulin receptor substrate (IRS).
From this perspective, we will review recent progress, highlight areas of uncertainty or controversy and suggest potential new mechanisms that may be involved in the cellular mechanisms of IR during CKD.

**Mechanisms of IR**

Insulin is an anabolic hormone whose main function is to control energy homeostasis by modulating carbohydrate and lipid metabolisms. Basically, insulin promotes the storage of glucose as glycogen in the liver and muscles and stimulates lipid storage as triglycerides in WAT (see Figure 1). In adipocytes and muscle cells, insulin activates the insulin receptor tyrosine kinase, which subsequently activates tyrosine phosphorylates IRS proteins. Once activated, these IRS activate phosphatidylinositol-3-kinase (PI3 K) providing a docking site for the p85 regulatory subunit and releasing the p110 catalytic subunit. PI3 K catalyzes the production of phosphatidylinositol [3, 4, 5]-trisphosphate (PIP3) from phosphatidylinositol [3, 4]-biphosphate (PIP2), which triggers phosphorylation of protein kinase B/Akt (PKB/Akt) and allows the translocation of insulin-sensitive glucose transporter (GLUT4) to the plasma membrane and facilitates the uptake of glucose (see Figure 2).

IR is characterized by resistance to the effects of insulin on glucose uptake, metabolism or storage and is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output. These functional defects may result from impaired insulin signalling in the target organs, which may be linked to reduced insulin binding to its receptor, blunted receptor phosphorylation, decreased tyrosine kinase activity and/or impaired phosphorylation of IRS proteins. A major mechanism by which insulin signalling can be negatively regulated is via phosphorylation of certain serine residues on IRS-1 [11, 14].

Several works over the last two decades have led to a unifying hypothesis that intracellular accumulation of toxic lipids species termed ‘lipotoxicity’ triggers activation of novel protein kinases C (PKC) with subsequent impairments in insulin signalling via inhibitory serine phosphorylation of IRS-1 [11]. It is now admitted in cases in which adipose tissue is absent, deficient or insulin resistant with a limited ability to store the energy excess, the triglyceride surplus will be deposited at undesirable sites, such as the liver, heart, skeletal muscle and in visceral adipose tissue, which is described as ectopic fat deposition. Indeed, accumulation of ectopic lipids such as diacylglycerols (DAGs) activates serine kinase such as PKC-θ in muscle and PKC-ɛ in liver. Additionally, other lipid derivatives such as ceramides can directly inhibit the phosphorylation of PKB/Akt [12]. Furthermore, while clues of the involvement of inflammation in diabetes trace date from a century ago, these last decade experiments showing that adipose tissue-derived proinflammatory cytokines such as TNF-α could actually cause IR in experimental models provided the first credible hypothesis linking inflammation to the development of IR [3]. An acute elevation of TNF-α in plasma causes an increased lipolysis, therefore increasing circulatory free fatty acid (FFA) levels [13]. More recently, experimental studies have suggested the contribution of gut microbiota to the development of obesity and related complications. The first pioneering experiments showed that colonization of ‘germ-free’ mice with an ‘obese microbiota’ results in a significantly greater increase in total body WAT and metabolic complications than colonization with a ‘lean microbiota’ [4]. The modification of the intestinal flora interferes with intestinal permeability, increasing the absorption of lipopolysaccharide (LPS) which are major components of the outer membrane of gram-negative bacteria. LPS activates inflammatory pathways by promoting expression of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs) after binding to Toll-like receptor (TLR) 4/2 and receptor CD14 in innate immune cells and adipocytes and increases the production of TNF-α and interleukin-6 (IL-6) [3]. Both inflammatory cytokines (TNF-α, IL-6) through activation of serine kinases such as Jun N-terminal kinase (JNK), NF-κB, mammalian target of rapamycin (mTOR) induce IR by increased inhibitory serine phosphorylation of IRS-1 [11, 14].

**IR in CKD**

CKD patients demonstrate a normal or mildly elevated glucose level during fasting and an enhanced increase of glucose following glucose loading. Patients may develop hyperglycaemia or maintain normoglycemia at the expense of hyperinsulinaemia [1], suggesting peripheral resistance to the action of insulin. These changes are often masked by a decline in the metabolic clearance of insulin that occurs as the glomerular filtration rate drops<15–20 mL/min [15].

As muscle tissue is the primary site for glucose disposal [16, 17] altered insulin sensitivity was considered to affect primarily muscle rather than liver. Friedman et al. [18] demonstrated that the increase in insulin-stimulated glucose transport was significantly reduced by 50% in muscles from uraemic patients without reduced glycogen synthase activity. However, the increase in hepatic glucose production (HGP) during uraemia is a subject of controversy. HGP measured during the hyperinsulinemic euglycemic clamp is reported to be similar to control subjects [1, 18]. However, the magnitude of HGP is too small and the difference between the groups may have been masked by the high insulin doses used in these experiments. Turnover studies using radiolabeled alanine and glucose have shown an increased glucose production in CKD subjects, which is mainly related to increased rate of gluconeogenesis from alanine which, in turn, is dependent on increased production of alanine in the post-absorptive state [20]. Thus, at higher physiological insulin concentrations, the inhibitory effect of insulin on HGP seems to be decreased in uraemia. Currently, there are no data on the effect of uraemia on adipose tissue insulin sensitivity in human. However, when incubated with serum from CKD patients, isolated rat adipocytes exhibit a decreased insulin stimulated glucose uptake and lipogenesis [18].

Several studies have investigated the cellular mechanisms of IR in CKD. In partially purified insulin receptors prepared from human [18] or rat [21] skeletal muscle, receptor number, insulin binding, β-subunit receptor auto-phosphorylation and
tyrosine kinase activity were all unchanged in CKD, suggesting that the IR observed in uraemic patients is due to a post-receptor defect of insulin pathway. Data on the abundance of GLUT4 in muscle are controversial. Cecchin et al. found no difference [21], while others reported a decreased expression of GLUT4 skeletal muscle in uraemic rats compared with controls [22]. Expression of several components of the insulin signalling pathway (IRS-1, PI3K) is not altered at the mRNA level in WAT from uraemic patients [23]. In muscles from 7/8 nephrectomized rats, Bailey et al. found functional abnormalities in the PI3K cascade with subsequent decreased activation of the downstream effector PKB/Akt. Interestingly, these abnormalities were overcome when the signalling pathway was maximally stimulated with supra-physiological dose of insulin [24]. Furthermore, the same group has shown a decreased tyrosine phosphorylation of IRS-1 and a decreased serine phosphorylation of PKB/Akt in muscle of CKD mice after insulin stimulation [25].

In contrast to obese or type 2 diabetes patients with normal renal function, serum from CKD patients inhibits insulin-stimulated glucose uptake in isolated rat adipocytes [26, 27]. Interestingly, incubation of skeletal muscle fibre strips from CKD patient, in a normal serum, improves basal glucose transport [18]. Taken together, these observations suggest that one or several unknown circulating molecules unique to uraemia could induce IR and downstream dysfunction of the IRS-PI3K-Akt pathway. However, numerous fractionation studies, which attempted to decipher the precise molecular nature of this factor, have so far not been conclusive. The components present in the uraemic serum and involved in IR in CKD are multiple, resulting from pathophysiological alterations related to CKD (acidosis, disturbed bone metabolism, accumulation of URMs, post-translational protein modification) and non-specific factors (dyslipidemia, systemic inflammation, oxidative stress …).

**ACIDOSIS AND IR IN CKD**

The concept that metabolic acidosis might be the cause of IR in CKD stems from both animal and human studies in which

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**FIGURE 1:** Insulin is the main regulator of carbohydrate metabolism. Insulin acts mainly on 3 target tissues to decrease blood glucose level: liver, skeletal muscle and WAT. When glycaemia is above the baseline value (>1 g/L), pancreatic beta cells secrete insulin. Insulin stimulates glucose uptake into muscle and white adipose cells and inhibits hepatic glucose production to return glycaemia to its baseline value.
ammonium chloride load induces IR. *In vitro*, an acidic pH is sufficient to reduce post receptor signalling through suppression of PKB/Akt phosphorylation in cultured muscle cells [28]. In muscle from 7/8 nephrectomized rats, the functional abnormalities in the insulin signalling pathway are partially restored with the correction of acidosis [24]. However, most of the experiments have been performed in severe acidosis conditions (serum pH ≈ 7.1), and a milder degree of acidosis might not exert the same effect. Finally, in human the degree of acidosis has been shown to correlate positively with the IR in CKD subjects [29].

**FIGURE 2:** Overview of the intracellular insulin signalling pathways in skeletal muscle and WAT. Insulin activates the insulin receptor kinase, which autophosphorylates and induces tyrosine phosphorylation of IRS-1, leading to activation of PI3K and production of PIP2 from PIP3. PIP3 activates PDK-1 that phosphorylates PKB/Akt on serine and threonine residues. PKB/Akt phosphorylates AS160 that stimulates translocation of glucose transporter GLUT-4 to plasma membrane and ultimately allows the uptake of glucose into the cells. GLUT-4 (glucose transporter-4) IRβ (insulin receptor beta subunit, IRS-1 (insulin receptor substrate-1) PDK-1 (3-phosphoinositide-dependent kinase), PI3-K (phosphoinositide-3 kinase) PIP2 (phosphatidylinositol 4,5-bisphosphate, PIP3 (phosphatidylinositol (3,4,5)-trisphosphate), PKB/Akt (protein kinase B/Akt).

VITAMIN D DEFICIENCY, HYPERPARATHYROIDISM AND IR IN CKD

There is evidence that the vitamin D and/or parathyroid hormone (PTH) axis is important in the pathogenesis of IR during uraemia. Several mechanisms have been proposed, including gene polymorphisms and the immunoregulatory function of vitamin D and inflammation. Vitamin D may have a beneficial effect on insulin action by stimulating the expression of insulin receptors enhancing insulin responsiveness for glucose transport [30]. After 4 weeks of intravenous 1,25-(OH)2 vitamin D3 therapy, IR is corrected in hemodialysis (HD) patients, in the absence of PTH suppression [31]. In addition to the classical actions of PTH on calcium metabolism, experimental data show that PTH plays a role in insulin sensitivity. PTH treatment of differentiated adipocytes suppresses insulin-stimulated glucose uptake and insulin signalling via cAMP pathway, potentially through the serine phosphorylation of IRS-1 [32]. PTH also stimulates HGP from lactate and pyruvate [33]. Finally, correction of secondary hyperparathyroidism in patients with CKD improves the glucose intolerance and insulin secretion [34].

PROTEIN CARBAMYLATION AND IR

A non-specific binding reaction between cyanic acid (CHNO) formed from urea and protein or peptide is called carbamylation. This reaction by changing the structure and charge of molecules can affect their biological function. Carbamylated proteins are increased with decreased renal function and associated with the alteration of metabolic pathways. In fact, insulin-sensitive glucose uptake is
recently reported that exposing C2C12 myotubes or important compound of protein-bound retention solutes. We toxins that are poorly removed by the common dialysis techni-
animal models of obesity, increased LPS absorption is due to decreased expression of tight junction (TJ) protein zonula occludens-1 (ZO-1) in the ileum, resulting in an increased gut permeability [45]. LPS activates IRS serine/threonine kinase through its binding to TLR4. Gut microbiota derived uraemic toxins (such as p-cresyl sulphate) disrupt insulin signalling through activation of IRS serine/threonine kinase such as ERK, DAG (diacylglycerols), ERK (extracellular signal-regulated kinase), FFA (free fatty acids), GSK3 (glycogen synthase kinase 3), IKK-β (inhibitor of nuclear factor kappa-β kinase subunit beta), IL6 (interleukin-6), IRS (insulin receptor substrate), JAK (Janus kinase), JNK (c-Jun N-terminal kinase), LPS (lipopolysaccharide), mTOR (mammalian target of rapamycin), PKB/Akt (protein kinase B/Akt), PKC (protein kinase C), TNF-α (tumour necrosis factor alpha), TLR4 (toll-like receptor 4), SOCS (suppressor of cytokine signalling), STAT (signal transducer and activator of transcription).

There is increasing evidence showing that CKD-associated disruption of intestinal epithelial barrier function is mediated by the retained uraemic toxins or metabolites. Vaziri et al. demonstrated in vitro study on human enterocytes that the heavy influx of URM in intestinal tract [49], especially urea and ammonium hydroxide generated from hydrolysis of urea by microbial urease [50] caused a significant reduction in the TJ proteins. These results are confirmed in animal studies, demonstrating that the adsorption of urea and ammonia and the reduction of entero-hepatic recycling of urea, through the administration of AST120 [47], attenuated CKD-induced disruption of the TJ proteins. Finally, the restoration of intestinal barrier in uraemic rats is also associated with a decrease in endotoxemia and inflammation [47]. These findings highlight a novel mechanism for the previously documented beneficial effect of urea-lowering strategies, e.g. low-protein diet and longer dialysis regimens in advanced CKD.
ADIPOSE TISSUE DYSFUNCTION: LIPOTOXICITY, INFLAMMATION AND OXIDATIVE STRESS IN CKD

WAT is now recognized as a major site for production of inflammatory cytokines and oxidative stress. These common features, which are present in the early stages of CKD, are associated with IR [51]. Moreover, clinical correlations have demonstrated that fat mass and abdominal subcutaneous fat are primary determinants of IR in ESRD patients [52] leading to the hypothesis that the uraemic milieu alters adipocyte physiology.

Several in vitro studies show that uraemic serum contributes to adipokines dysregulations, systemic inflammation and oxidative stress in adipocytes cells. Indeed we demonstrated that human uraemic plasma induces exuberant secretion of leptin in 3T3-L1 adipocytes [53]. Moreover some uraemic toxins such as urea could induce ROS production and increase expression of adipokines retinol binding protein-4 (RBP-4) and resistin [8]. Subcutaneous WAT from subjects with advanced CKD shows an increased expression of the immune cell marker CD68, IL-6 and suppressor of cytokine signalling-3 (SOCS-3) and oxidative stress genes (Uncoupling protein 2 and cytochrome b-245, alpha polypeptide), suggesting that CKD is associated with increased adipose tissue inflammation [23, 54].

Oxidative stress and inflammation are inseparably linked, as they form a vicious cycle in which oxidative stress provokes inflammation by several mechanisms including activation of the transcription factor NF-κB, which leads to the activation of immune cells. Inflammation, in turn, triggers oxidative stress via production of ROS by the activated leukocytes. It is now well accepted that increased ROS levels are an important trigger for IR [55]. Nuclear factor-erythroid 2-related factor 2 (Nrf2) is the master regulator of the cellular adaptive response to oxidative stress and recognized to be pivotal for the induction of several genes encoding antioxidant and anti-inflammatory proteins. Nrf2 knock out ob/ob mice show reduced WAT mass but develop a more severe metabolic syndrome and IR [56]. Animal models of CKD exhibit a marked decline in Nrf2 activity along with the reduction of its target genes products in the remnant kidney. The effect of CKD on the expression of Nrf2 in other insulin sensitive tissues has not been investigated yet. Given its critical role, Nrf2-targeted strategies may hold promise for prevention of IR in CKD [57]. In addition to the low-grade inflammation, experimental data suggest that uraemia induces blunted lipogenesis and/or increased lipolysis, which could promote lipid redistribution in the body and in turn lipotoxicity. Recently, Axelson et al. reported that uraemic sera from CKD stage 5 patients stimulate basal lipolysis in cultured human adipocytes [58]. Similarly, we confirm this result showing that uraemic serum stimulates basal lipolysis and significantly decreases lipogenesis in 3T3-L1 preadipocytes [59]. Interestingly, pre-treatment of these cells with p-cresyl sulphate have the same effect as the uraemic serum [9]. Zhao et al. reported that uninephrectomized rats exhibit a progressive loss of WAT associated with ectopic lipid deposition and IR [10]. Similarly, we reported that 5/6 subtotal nephrectomized rats [59] and mice [9] exhibited lipoatrophy and ectopic lipid redistribution in muscle and liver. Jin et al. have showed that uraemic rats exhibit an increased expression of a master regulator of lipogenesis (ChREBP, carbohydrate response element binding protein), associated with a downregulation of peroxisome proliferator-activated receptor-α (PPARα) regulated fatty acid oxidation system and reduction of diacylglycerol acyltransferase (DGAT) resulting in reduced fatty acid incorporation in triglycerides. However, the available published data do not clearly identify the primary dysfunction leading to ectopic lipid accumulation [60]. Moreover, the nature of the lipids accumulated in these models is unknown and this phenomenon remains to be demonstrated in humans. One hypothesis invoked to explain the accumulation of toxic lipids (such as ceramides or DAG) is mitochondrial dysfunction. In fact, subtotal nephrectomized rats show a reduction of mitochondrial oxidative capacity, as reflected by representative mitochondrial enzyme activities such as peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, PGC-1β, mitochondrial transcription factor-A (mTFA), and peroxisome proliferator activated receptor-α [61]. Copy number of mitochondrial DNA are reported to be lower among dialysis patients compared with healthy subjects [62].

CONCLUSION

The aetiology of IR in CKD is multifactorial and includes a complex network including inflammation, adipokines, lipotoxicity and uraemic toxins, leading to an acquired defect of the insulin receptor-signalling pathway, especially via the inhibition of IRS-1 through serine residue phosphorylation (see Figure 3). The expanding DNA/RNA sequencing technologies and knowledge of the chemical identity of the URMs have allowed us to make a step forward linking microbial-generated toxins to CKD-induced IR. This is of particular interest, since their production may prove simpler to suppress than the production of other waste solutes. Furthermore, the increasing knowledge of adipose tissue biology and its role in the regulation of energy metabolism have given rise to a unifying hypothesis such as the phenomenon of lipotoxicity linking ectopic lipid accumulation and IR. Although convincing in animal studies, whether dysbiosis or ectopic lipid accumulation is the cause of IR needs to be confirmed in human CKD subjects.

ACKNOWLEDGEMENTS

L.K. held a fellowship from Fondation pour la Recherche Médicale and. C.P. and P.A. held a grant from Société Francophone de Néphrologie. This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM) and Institut National des Sciences Appliquées de Lyon (INSA-Lyon). The authors gratefully acknowledge Dr Nicolas J Pillon (The Hospital for Sick Children, University of Toronto, Canada) who kindly allows them the free use of his illustrations.
CONFLICT OF INTEREST STATEMENT

None declared.

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Received for publication: 15.5.2013; Accepted in revised form: 7.9.2013